



Faculty of Resource Science and Technology

**Secondary Metabolites from *Garcinia dryobalanoides* and
their Anti-bacterial Activity**

Nur Fazlin Zafirah binti Zaine

**Master of Science
2024**

Secondary Metabolites from *Garcinia dryobalanoides* and their
Anti-bacterial Activity

Nur Fazlin Zafirah binti Zaine

A thesis submitted

In fulfillment of the requirements for the degree of Master of Science

(Organic Chemistry)

Faculty of Resource Science and Technology

UNIVERSITI MALAYSIA SARAWAK

2024

DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

.....

Signature

Name: Nur Fazlin Zafirah binti Zaine

Matric No.: 22020211

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

Date : 25/04/2024

ACKNOWLEDGEMENT

First and foremost, praises and thanks to Allah the Almighty, for His showers of blessings throughout all the work to complete this study.

Next, I would like to express my deep and sincere gratitude to my supervisor Dr. Nor Hisam binti Zamakshshari for giving me invaluable guidance and support throughout completing this project. Her dynamism, vision, sincerity, and motivation have deeply inspired me a lot. It was a great privilege and honor to study under her guidance. I am extremely grateful for what she has offered me. Along with that, I would like to thank Dr. Vivien, Dr. Rosmawati and Dr. Ainaa Nadiah for motivation and willingness to share their knowledge which help me a lot throughout this research.

Besides that, I would like to thank my laboratory partner, senior, and friends, especially Anisa, Mas Atikah, Hadira, Aina, Kai Wei, Davlye for their assistance, encouragement, and support throughout this research period. In addition, my warmest thanks to all the science officers as well as the lab assistants for their exemplary guidance.

Also, I would like to express my heartfelt appreciation and gratitude to my family, for their love, care, encouragement, and continued prayers for my success in completing this research.

Last but not least, I want to thank me. I want to thank me for believing in me, I want to thank me for doing all this hard work, I want to thank me for having no days off, I want to thank me for never quitting, I want to thank me for always being a giver and trying to give more than I receive. I want to thank me for doing more right than wrong. I want to thank me for just being me at all time.

ABSTRACT

Garcinia was discovered to have excellent sources of bioactive compounds with therapeutic potential and hence received significant attention due to its potential for medicinal applications. This study investigates *Garcinia* species' secondary metabolites and their anti-bacterial activity, specifically *Garcinia dryobalanoides*. Different chromatographic methods were used to isolate and purify the pure compounds. Along with that, structural modification of the major compound was successfully synthesized *via* Williamson etherification. The structural elucidations of these isolated and synthesized compounds were achieved through FT-IR, UV-Vis, LC-MS, 1D, and 2D-NMR. Anti-bacterial tests were conducted against various bacterial strains, including Gram-positive bacteria (*Lactiplantibacillus plantarum*) and Gram-negative bacteria (*Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Serratia marcescens*). Six compounds (**76**, **2**, **77**, **78**, **79** and **59**) were successfully isolated and five rubraxanthone derivatives (**80** – **84**) were successfully synthesized. Astonishingly, compound **76** was the first naturally occurring benzophenone isolated from the *Garcinia* genus. The anti-bacterial evaluation on crude extract, isolated, and synthesized compounds against tested bacteria demonstrated moderate to excellent activities outlining anti-bacterial potential. Structure-activity relationships (SAR) study demonstrated that xanthenes bearing prenyl and hydroxy groups exhibit excellent bacterial inhibition. However, adding an alkyl chain to the hydroxyl group reduced this effect, possibly due to steric hindrance. This study highlights *Garcinia* species as a promising source of natural anti-bacterial and further investigation is needed.

Keywords: *Garcinia dryobalanoides*, secondary metabolites, extract, anti-bacterial activity, xanthone derivatives

Metabolit Sekunder daripada Garcinia dryobalanoides dan Aktiviti Anti-bakteria

ABSTRAK

Garcinia didapati mempunyai sumber sebatian bioaktif yang sangat baik dengan nilai terapeutik yang tinggi. Oleh itu, ia telah mendapat perhatian kerana potensinya dalam bidang perubatan. Kajian ini dijalankan bagi menyiasat metabolit sekunder dan aktiviti anti-bakteria dari spesies *Garcinia*, khususnya *Garcinia dryobalanoides*. Pelbagai kaedah kromatografi telah digunakan dalam mengisolasi dan purifikasi sebatian kimia tulen dari spesies ini. Selain itu, pengubahsuaian struktur sebatian kimia utama berjaya disintesis melalui Williamson etherifikasi. Pengenalan struktur bagi sebatian kimia tulen telah dicapai melalui FT-IR, UV-Vis, LC-MS, 1D, dan 2D-NMR. Ujian anti-bakteria telah dijalankan terhadap pelbagai strain bakteria, termasuk bakteria Gram-positif (*Lactiplantibacillus plantarum*) dan bakteria Gram-negatif (*Enterobacter cloacae*, *Pseudomonas aeruginosa*, dan *Serratia marcescens*). Enam sebatian kimia tulen (76, 2, 77, 78, 79 dan 59) berjaya dipurifikasi dan lima derivatif rubraxantone (80 – 84) berjaya disintesis. Sebatian 76 ialah benzofenon yang pertama terhasil secara semula jadi yang diasingkan daripada genus *Garcinia*. Penilaian anti-bakteria ke atas ekstrak *G. dryobalanoides*, sebatian tulen dan sintesis, terhadap bakteria yang diuji menunjukkan aktiviti antibakteria yang sederhana dan tinggi. Kajian SAR menunjukkan bahawa xanton yang mengandungi kumpulan prenil dan hidroksi mempamerkan perencatan bakteria yang sangat baik. Walau bagaimanapun, penambahan rantai alkil kepada kumpulan hidroksil mengurangkan kesan ini, mungkin disebabkan oleh halangan sterik. Kajian ini telah membuktikan spesies *Garcinia* sebagai sumber anti-bakteria semulajadi yang bagus dan penyelidikan lebih mendalam amat diperlukan.

Kata kunci: Garcinia dryobalanoides, *metabolit sekunder, ekstrak, aktivitas anti-bakteria, xanton derivatif*

TABLE OF CONTENTS

	Page
DECLARATION	i
ACKNOWLEDGEMENT	ii
ABSTRACT	iii
<i>ABSTRAK</i>	iv
TABLE OF CONTENTS	vi
LIST OF TABLES	xi
LIST OF FIGURES	xii
LIST OF SCHEMES	xvi
LIST OF ABBREVIATIONS	xvii
CHAPTER 1: INTRODUCTION	1
1.1 Background of Study	1
1.2 Genus <i>Garcinia</i>	2
1.3 Problem Statement	4
1.4 Objectives of Study	5
CHAPTER 2: LITERATURE REVIEW	7
2.1 Secondary Metabolite	7
2.2 Secondary Metabolite from <i>Garcinia sp.</i>	9

2.2.1	Xanthones	11
2.2.2	Flavonoids	14
2.2.3	Biflavonoids	16
2.2.4	Other Secondary Metabolites in <i>Garcinia</i>	17
2.3	Structural Modification of Xanthone	22
2.4	Anti-microbial Activity of <i>Garcinia</i> Genus	23
2.4.1	Anti-microbial Activity of <i>Garcinia</i> Crude Extract	25
2.4.2	Anti-microbial Activity of <i>Garcinia</i> Secondary Metabolites	28
CHAPTER 3: MATERIALS AND METHODOLOGY		34
3.1	Plant Materials	34
3.2	Chemicals and Reagents	34
3.2.1	Extraction	34
3.2.2	Isolation and Purification	34
3.2.3	Instrumental Analysis	35
3.2.4	Anti-bacterial Assay	35
3.3	Extraction, Isolation and Purification of Secondary Metabolites	35
3.3.1	Extraction, Isolation and Purification	35
3.3.2	Chromatographic Method	36
3.3.2.1	Column Chromatography (CC)	36
3.3.2.2	Thin Layer Chromatography (TLC)	36

3.4	Structural Elucidation and Characterization	36
3.5	Physical and Spectral Data of Secondary Metabolites from <i>Garcinia dryobalanoides</i>	37
3.5.1	2-Hydroxy-(4-octyloxy)benzophenone (76)	37
3.5.2	Rubraxanthone (2)	38
3.5.3	8-Deoxygartanin (77)	39
3.5.4	Isocowanol (78)	40
3.5.5	9-Hydroxycalabaxanthone (79)	41
3.5.6	Stigmasterol (59)	42
3.6	Structural Modification of Rubraxanthone (2)	43
3.6.1	3- <i>O</i> -propyl-rubraxanthone (80)	44
3.6.2	6- <i>O</i> -propyl-rubraxanthone (81)	45
3.6.3	3,6- <i>O</i> -pentyl-rubraxanthone (82)	46
3.6.4	3,6- <i>O</i> -hexyl-rubraxanthone (83)	47
3.6.5	6- <i>O</i> -butyl-rubraxanthone (84)	48
3.7	Anti-bacterial Assay	48
3.7.1	Agar Well Diffusion Method	48
3.7.2	Minimum Inhibition Concentration (MIC)	49
3.7.3	Minimum Bactericidal Concentration (MBC)	50
3.8	Statistical Analysis	50

CHAPTER 4: RESULTS AND DISCUSSION	51
4.1 Extraction and Isolation of Secondary Metabolites from <i>Garcinia dryobalanoides</i>	51
4.2 Characterization of Secondary Metabolites from <i>Garcinia dryobalanoides</i>	52
4.2.1 Characterization of 2-Hydroxy-(4-octyloxy)benzophenone (76)	52
4.2.2 Characterization of Rubraxanthone (2)	59
4.2.3 Characterization of 8-Deoxygartanin (77)	65
4.2.4 Characterization of Isocowanol (78)	70
4.2.5 Characterization of 9-hydroxycalabaxanthone (79)	76
4.2.6 Characterization of Stigmasterol (59)	82
4.3 Structural Modification of Rubraxanthone (2)	86
4.3.1 Characterization of 3- <i>O</i> -propyl-rubraxanthone (80) and 6- <i>O</i> -propyl-rubraxanthone (81)	86
4.3.2 Characterization of 3,6- <i>O</i> -pentyl-rubraxanthone (82)	97
4.3.3 Characterization of 3,6- <i>O</i> -hexyl-rubraxanthone (83)	103
4.3.4 Characterization of 6- <i>O</i> -butyl-rubraxanthone (84)	109
4.4 Anti-bacterial Assay	113
4.4.1 Agar Well Diffusion Method	113
4.4.2 Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC)	117
4.4.3 Structure-activity relationship (SAR) studies	118

CHAPTER 5:CONCLUSION AND RECOMMENDATIONS	121
5.1 Conclusion	121
5.2 Recommendations	122
REFERENCES	123
APPENDICES	139

LIST OF TABLES

	Page
Table 2.1 Plant kingdom phenolic class	10
Table 4.1 1D and 2D-NMR assignments of 2-hydroxy-(4-octyloxy)benzophenone (76)	55
Table 4.2 1D and 2D-NMR assignments of rubraxanthone (2)	63
Table 4.3 1D and 2D-NMR assignments of 8-deoxygartanin (77)	68
Table 4.4 1D and 2D-NMR assignments of isocowanol (78)	74
Table 4.5 1D and 2D-NMR assignments of 9-hydroxycalabaxanthone (79)	80
Table 4.6 ¹ H NMR and ¹³ C NMR assignments of stigmasterol (59)	84
Table 4.7 1D and 2D-NMR assignments of 3- <i>O</i> -propyl-rubraxanthone (80)	91
Table 4.8 1D and 2D-NMR assignments of 6- <i>O</i> -propyl-rubraxanthone (81)	94
Table 4.9 1D and 2D-NMR assignments of 3,6- <i>O</i> -pentyl-rubraxanthone (82)	100
Table 4.10 1D and 2D-NMR assignments of 3,6- <i>O</i> -hexyl-rubraxanthone (83)	106
Table 4.11 ¹ H NMR assignments of 6- <i>O</i> -butyl-rubraxanthone (84)	111
Table 4.12 Anti-microbial activity of plant extract	114
Table 4.13 Anti-microbial activity of isolated and synthesized compound	116

LIST OF FIGURES

	Page
Figure 1.1 Herbarium specimen of leaves and twigs of <i>G. dryobalanoides</i>	3
Figure 2.1 Origin of the main categories of secondary metabolites	8
Figure 2.2 Xanthoness isolated from <i>G. fagraeoides</i> and <i>G. mangostana</i>	12
Figure 2.3 Xanthoness isolated from <i>G. nigrolineata</i> , <i>G. nervosa</i> and <i>G. cowa</i>	13
Figure 2.4 Xanthoness isolated from <i>G. tetrandra</i>	14
Figure 2.5 3-C bridge (C ₃ C ₆ C ₃) of flavonoids	15
Figure 2.6 Flavonoids isolated from <i>Garcinia</i> species	15
Figure 2.7 Biflavonoids isolated from <i>Garcinia</i> species	16
Figure 2.8 Coumarin isolated from <i>Garcinia</i> species	17
Figure 2.9 Benzoquinones isolated from <i>Garcinia</i> species	18
Figure 2.10 Phloroglucinols isolated from <i>Garcinia</i> species	19
Figure 2.11 Benzophenones isolated from <i>Garcinia</i> species	20
Figure 2.12 Terpenes isolated from <i>Garcinia</i> species	21
Figure 2.13 Phenolic compounds possessed anti-bacterial activity	30
Figure 4.1 Secondary metabolites isolated from <i>G. dryobalanoides</i>	52
Figure 4.2 LC-MS of compound 76	52
Figure 4.3 FT-IR of compound 76	53
Figure 4.4 HMBC and COSY correlation in compound 76	55
Figure 4.5 ¹ H NMR of compound 76	56
Figure 4.6 ¹³ C NMR of compound 76	56
Figure 4.7 HSQC spectra of compound 76	57

Figure 4.8	DEPT spectra of compound 76	57
Figure 4.9	COSY spectra of compound 76	58
Figure 4.10	HMBC spectra of compound 76	58
Figure 4.11	LC-MS of compound 2	59
Figure 4.12	FT-IR of compound 2	60
Figure 4.13	HMBC correlation in compound 2	62
Figure 4.14	¹ H NMR of compound 2	64
Figure 4.15	¹³ C NMR of compound 2	64
Figure 4.16	LC-MS of compound 77	65
Figure 4.17	FT-IR of compound 77	66
Figure 4.18	HMBC and COSY correlation in compound 77	68
Figure 4.19	¹ H NMR of compound 77	69
Figure 4.20	¹³ C NMR of compound 77	69
Figure 4.21	LC-MS of compound 78	70
Figure 4.22	FT-IR of compound 78	71
Figure 4.23	HMBC correlation in compound 78	73
Figure 4.24	¹ H NMR of compound 78	75
Figure 4.25	¹³ C NMR of compound 78	75
Figure 4.26	LC-MS of compound 79	76
Figure 4.27	FT-IR of compound 79	77
Figure 4.28	HMBC correlation in compound 79	79
Figure 4.29	¹ H NMR of compound 79	81
Figure 4.30	¹³ C NMR of compound 79	81
Figure 4.31	LC-MS of compound 59	82

Figure 4.32	FT-IR of compound 59	83
Figure 4.33	¹ H NMR of compound 59	85
Figure 4.34	¹³ C NMR of compound 59	85
Figure 4.35	LC-MS of compound 80	87
Figure 4.36	LC-MS of compound 81	87
Figure 4.37	FT-IR of compound 80	88
Figure 4.38	FT-IR of compound 81	89
Figure 4.39	HMBC correlation in compound 80	91
Figure 4.40	¹ H NMR of compound 80	92
Figure 4.41	¹³ C NMR of compound 80	92
Figure 4.42	HMBC spectrum of compound 80	93
Figure 4.43	HMBC correlation in compound 81	94
Figure 4.44	¹ H NMR of compound 81	95
Figure 4.45	¹³ C NMR of compound 81	95
Figure 4.46	HMBC spectrum of compound 81	96
Figure 4.47	LC-MS of compound 82	97
Figure 4.48	FT-IR of compound 82	98
Figure 4.49	HMBC correlation in compound 82	99
Figure 4.50	¹ H NMR of compound 82	101
Figure 4.51	¹³ C NMR of compound 82	101
Figure 4.52	HMBC spectrum of compound 82	102
Figure 4.53	LC-MS of compound 83	103
Figure 4.54	FT-IR of compound 83	104
Figure 4.55	HMBC spectrum of compound 83	105

Figure 4.56	¹ H NMR of compound 83	107
Figure 4.57	¹³ C NMR of compound 83	107
Figure 4.58	HMBC spectrum of compound 83	108
Figure 4.59	LC-MS of compound 84	109
Figure 4.60	FT-IR of compound 84	110
Figure 4.61	¹ H NMR spectrum of compound 84	112
Figure 4.62	Structure-activity relationships of xanthones	120

LIST OF SCHEMES

	Page
Scheme 2.1 Structural modification of ananixanthone	23
Scheme 3.1 Structural modification of rubraxanthone (2)	43

LIST OF ABBREVIATIONS

NMR	Nuclear Magnetic Resonance
<i>s</i>	singlet
<i>d</i>	doublet
<i>dd</i>	doublet of doublet
<i>t</i>	triplet
<i>m</i>	multiplet
1D-NMR	One-dimensional Nuclear Magnetic Resonance
¹ H NMR	Proton Nuclear Magnetic Resonance
¹³ C NMR	Carbon Nuclear Magnetic Resonance
DEPT	Distortionless Enhancement by Polarization Transfer
2D-NMR	Two-dimensional Nuclear Magnetic Resonance
COSY	Correlated Spectroscopy
HSQC	Heteronuclear Single Quantum Coherence
HMBC	Heteronuclear Multiple Bond Correlation
UV–vis	Ultraviolet-Visible Spectroscopy
FT-IR	Fourier Transform Infrared Spectroscopy
LCMS	Liquid Chromatography Mass- Spectrometry
DMSO	Dimethyl sulfoxide
TLC	Thin Layer Chromatography
IZD	Inhibition zone diameter
MIC	Minimum Inhibition Concentration
MBC	Minimum Bactericidal Concentration

GDH	<i>Garcinia dryobalanoides</i> hexane extract
GDEA	<i>Garcinia dryobalanoides</i> ethyl acetate extract
GDM	<i>Garcinia dryobalanoides</i> methanol extract
MRSA	<i>Methicillin-Resistant Staphylococcus aureus</i>
MDRS	<i>Multidrug-Resistant Salmonella</i>
SARs	Structure-activity relationship (SAR) studies

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The rise in infectious disease cases linked to anti-microbial resistance is a wake-up call for new anti-microbial agents to be discovered (Naves et al., 2019). The breakthrough of antibiotics, led by penicillin, resulted in a monumental paradigm shift in treating bacterial infections, substantially decreasing morbidity and mortality rates associated with infectious diseases (Liu et al., 2022). The medical community once hailed the discovery of antibiotics as a victory in the war against infectious diseases (Reygaert, 2018). However, misuse of the compound has increased bacterial resistance (Joana et al., 2019).

Notably, natural products (NPs) are a cornerstone of inspiration for innovative drug design and development (Davison & Brimble, 2019). Plants with medicinal benefits have recently piqued the interest of science and the pharmaceutical industry, of which natural substances account for approximately 73% of the drugs produced by this industry (Conceição et al., 2023). This is due to plants can produce secondary metabolites to resist pathogens, making them a promising source of anti-microbial leads (Liu et al., 2022). Moreover, investigating plants with medicinal properties yields valuable insights applicable to developing novel plant-based drugs and other therapeutic agents (Santo et al., 2020). Conversely, modifying the structure of the natural resources is also known to lead to novel medications (Yao et al., 2017). Several studies, including those by Lee et al. (2018), Zou et al. (2013), and Karunakaran et al. (2018), have investigated the modified structure of natural resources and evaluated their resulting biological activities which have led to new therapeutic agents.

Santo et al. (2020) stated that the former Guttiferae and Clusiaceae families stood out among all medicinal plants. Clusiaceae is a family of medicinal plants that includes approximately 50 genera and 600 species (Lim et al., 2021). It can be found in subtropical and tropical areas (Conceição et al., 2023). The Clusiaceae family is a diverse group of plants that includes lianas, woody perennials, shrubs, and trees (Mañourová et al., 2019). This family has been widely used in ethnomedicine as a remedy for various disease conditions, such as infection, inflammation, cancer, dysentery, ulcers, and wounds (Lim et al., 2021). *Garcinia*, is a plant genus that belongs to the Clusiaceae family (Santo et al., 2020), also received great attention due to its potential for a wide range of medicinal properties (Wong et al., 2017). The *Garcinia's* medicinal properties are attributed to its ample secondary metabolites (Wong et al., 2017), which include triterpenes, xanthones, flavonoids, coumarins, benzophenones, anthrones, and anthraquinones (Brito et al., 2017).

1.2 Genus *Garcinia*

Garcinia (synonym = *Rheedia*) (Gontijo et al., 2012) is a member of the Clusiaceae family, which was previously known as Guttiferae that can be found in Asia, Polynesia, Africa, and Australia (Akongwi et al., 2023). The genus was given its name after Laurent Garcin, a Swiss botanist who worked for the Dutch East India Company and released the first report on *Garcinia mangostana* (mangosteen), the most well-known fruit species in the genus of *Garcinia* (Mañourová et al., 2019). *Garcinia* is a dioecious evergreen tree (Paul & Zaman, 2022) that contains over 600 species (Akongwi et al., 2023), consists of leathery leaves, solitary flowers, and two to eight pulpy seeds with fleshy fruits (Paul & Zaman, 2022). The colour of the inner bark of *Garcinia* is occasionally white or yellow. Meanwhile, stembark can be either grey or brown. *Garcinia* branches and stems

exude “gamboge” (a gummy resin exudate). The colours of the exudates (cream, white, or yellow) play a role in figuring out the different *Garcinia* species (Paul & Zaman, 2022).

G. dryobalanoides (Figure 1.1) is one of the *Garcinia* species that grows in Malaysia. It is known as *kandis* by the locals. This botanical record, initially documented in the fifth volume of Flore Forestière de Cochinchine on page seven during the year 1883, designates the species' native plant as Borneo, Malaysia. This tree grows primarily within the wet tropical biome (POWO, 2024).



Figure 1.1: Herbarium specimen of leaves and twigs of *G. dryobalanoides*

Garcinia has been used in traditional medicine worldwide (Brito et al., 2017). The locals in regions where *Garcinia* species grow have used these compounds to heal various illnesses (Conceição et al., 2023). The leaves and stem bark of *G. fagraeoides* have been used to treat malaria and dermatitis in local traditional medicine (Thuy et al., 2022). Meanwhile, the bark of *G. kola* has been used to treat malaria and abdominal pain (Mañourová et al., 2019). *Garcinia* fruit infusions, on the other hand, were used to treat dysentery, ulcers and wounds (Santo et al., 2020). Aside from that, in Ayurvedic medicine, the fruits of *Garcinia* were used to treat infections and urinary disorders as well as to improve digestion. *Garcinia* gummy exudates as well are used as cathartics and emetics in folk medicine. For instance,

G. dulcis gummy exudates are used as an expectorant and laxative in Thai medicine (Paul & Zaman, 2022).

Conversely, the pharmaceutical industry has taken notice of the use of *Garcinia* plants to treat a variety of disorders (Brito et al., 2017). The Guttiferae family contains a variety of biologically active metabolites that have been shown to have a variety of intriguing biological properties such as anti-oxidant activity, anti-aflatoxigenic activity, anti-depressant activity, trypanocidal activity, anti-bacterial activity, cytotoxic activity, and anti-malarial activity (Gontijo et al., 2012). Khamthong and Hutadilok-Towatana (2017) also reported that several pharmacological and phytochemical studies of *Garcinia* species have revealed that the plant's various parts contain a diverse array of secondary metabolites, a great number of which have medicinal benefits.

1.3 Problem Statement

Nowadays, anti-microbial resistance (AMR) has become one of the crucial public health problems due to the emergence and spread of drug-resistant pathogens. The advent of β -lactam antibiotics, beginning with penicillin, has been instrumental in treating bacterial infections (Breijyeh et al., 2020). β -lactams were the most used anti-microbial agents (Reygaert, 2018). However, overuse and misuse of anti-microbial medications make infections more difficult to treat and potentially fatal (Liu et al., 2022). Thus, there's an urge to discover new compounds for anti-microbial research. Natural products have been an important framework for developing antibiotics. The genus *Garcinia*, for instance, has garnered significant attention due to its potential for diverse medicinal properties, particularly its anti-microbial activity (Wong et al., 2017). Malaysia has a tropical rainforest that consists of diverse tree species. However, many species, including Malaysian *Garcinia*

plants, have not yet been fully discovered. Given the limited research on *G. dryobalanoides*, its phytochemical properties and anti-microbial activity were investigated as part of the search for novel natural anti-microbial agents. Aside from that, the structural modification was done on the major compound isolated from *G. dryobalanoides*, rubraxanthone. Rubraxanthone has exhibited substantial anti-bacterial efficacy in previous research by Muhammad et al. (2019). By altering its structure, it was envisaged to enhance its anti-bacterial potency as earlier studies on synthesized compounds derived from natural sources have shown promising efficacy as potential alternatives to traditional antibiotics in combating multidrug-resistant infections (Elmaidomy et al., 2022).

1.4 Objectives of Study

In general, this study aimed to investigate the phytochemical and anti-bacterial activity of new *Garcinia* species. *Garcinia* species is known for their anti-microbial activity. *G. dryobalanoides* collected from Sarawak was selected for phytochemical and anti-bacterial studies. Besides isolation, structural modification of a major compound, rubraxanthone (**2**), was conducted to improve the anti-microbial properties owing to the presence of an alkyl chain for lipophilicity.

The specific objectives of this study were:

- i. To isolate, identify and characterize the secondary metabolites from *G. dryobalanoides* using various spectroscopic techniques.
- ii. To carry out the structural modification and characterization of rubraxanthone isolated from *G. dryobalanoides*.

- iii. To screen the anti-bacterial activity of crude extracts, isolated and synthesized compounds against Gram-positive and Gram-negative strains.

CHAPTER 2

LITERATURE REVIEW

2.1 Secondary Metabolite

Metabolites are small molecule intermediates in natural metabolic processes (Cooper & Nicola, 2014). Metabolite components, such as primary metabolites and secondary metabolites, can be classified based on their plant metabolism activity. Primary metabolites consist of proteins, chlorophyll, amino acids and sugar. Meanwhile, secondary metabolites comprise phenolic compounds, tannins, saponins, flavonoids, alkaloids and many more (Agidew, 2022).

According to Waksmundzka-Hajnos et al. (2008), all plants contain primary metabolites regardless of the plant family, species, or genus, as the metabolites are essential for plant life. Cooper and Nicola (2014) stated that primary metabolites are essential for plant reproduction, development, and growth. Secondary metabolites, on the other hand, are a wide range of compounds produced by plant cells, which are specifically produced *via* secondary metabolic pathways (Gorlenko et al., 2020). These pathways diverge from primary metabolic pathways required for plant growth and development. Meanwhile, secondary metabolites are significant in defense and interspecies competition, such as defending plants from microbes and herbivores (Gorlenko et al., 2020).

Over the past 50 years, there have been more research investigations on secondary metabolites of plants (Dey et al., 2017). Approximately 200,000 different secondary metabolites have been identified and isolated based on their biosynthesis pathways and/or chemical structure (Gorlenko et al., 2020). This is not surprising as secondary metabolites

are extraordinarily diverse, with several major classes containing thousands of compounds in each class. Secondary metabolites can be categorized according to their composition, chemical structure, biosynthetic pathway, or solubility in different solvents (Waksmundzka-Hajnos et al., 2008). The origin of the major categories of secondary metabolites in relation to the basic metabolic pathways in their report, depicted in Figure 2.1.

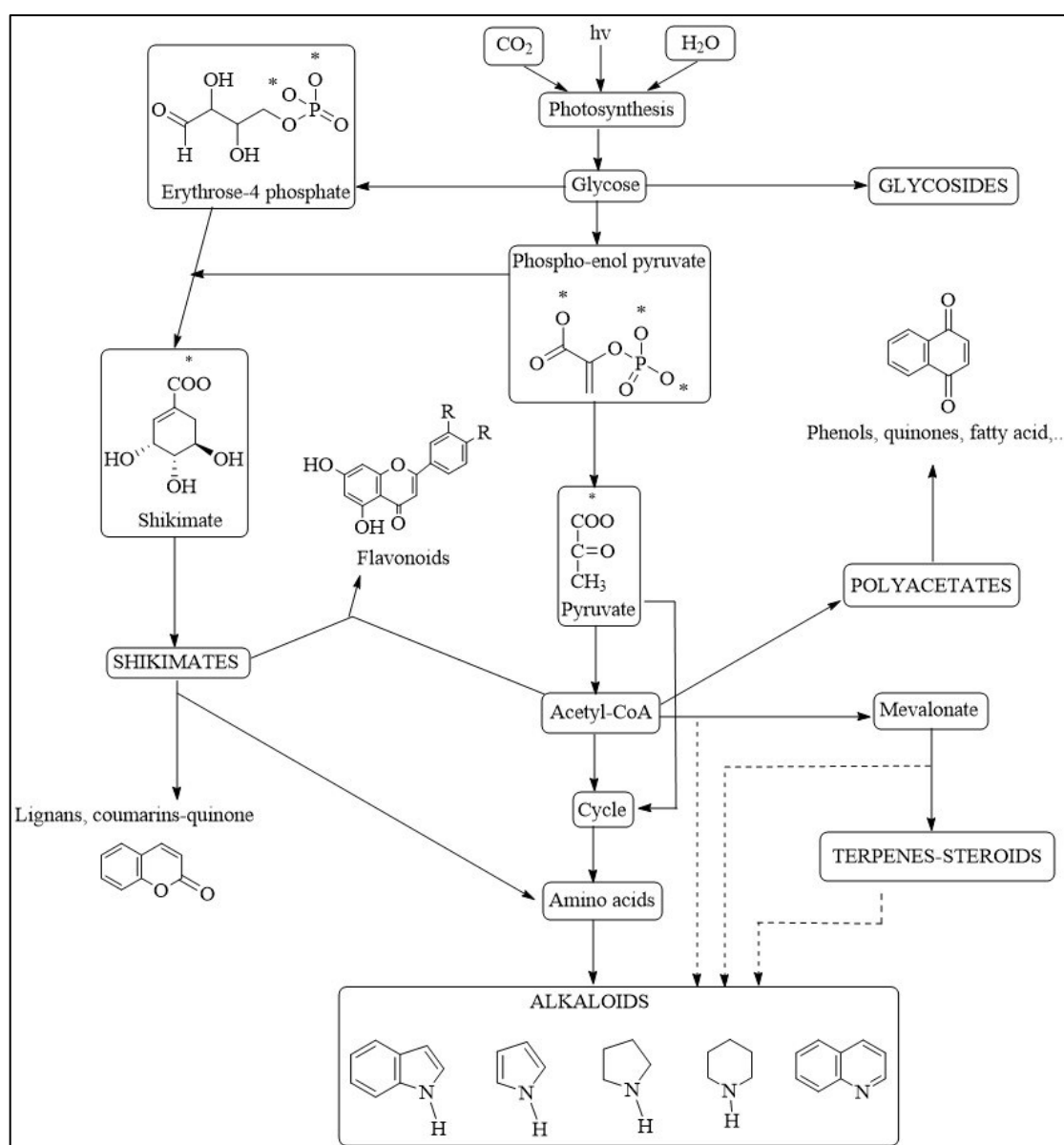


Figure 2.1: Origin of the main categories of secondary metabolites adapted from Waksmundzka-Hajnos et al. (2008)

Chromatographic techniques are commonly used to obtain these secondary metabolites and/or biologically active compounds. Column chromatography is one of the most common and well-known techniques for separating both inorganic and organic compounds. This implies that it could be useful in the chemical analysis of complex extract materials (Bajpai et al., 2016). In addition to methods above, several other techniques have been used to identify secondary metabolites, including Nuclear Magnetic Resonance (NMR), Mass Spectroscopy (MS), Ultraviolet-Visible Spectroscopy (UV-*vis*), Infrared Spectroscopy (IR), High-Resolution Electron Ionisation Mass Spectroscopy (HR-EI-MS), Electron-Ionisation Mass Spectroscopy (EI-MS) and High-Performance Liquid Chromatography (HPLC). These techniques are applicable to assess secondary metabolites' purity and quantify their concentration. Likewise, it is useful to recognize secondary metabolites based on their chemical structure, molecular weight, and functional groups (Velu et al., 2018).

Research on plant secondary metabolites as a source of drug discovery is a rapidly growing field. The most common secondary metabolites that have been studied for drug discovery include benzophenones, biflavonoids, coumarins, xanthonenes, tannins, phenols, steroids, and flavonoids (Dey et al., 2017). In the Clusiaceae family, benzophenones, biflavonoids, xanthonenes and coumarins are primarily produced as a defence mechanism (Brito et al., 2017). Many other secondary metabolites were also reported in this family, especially from the genus *Garcinia*.

2.2 Secondary Metabolite from *Garcinia* sp.

Garcinia has been identified to contain an extensive array of biologically active metabolites and its extracts are rich in these molecules (Lim et al., 2021). The branches,

latex, flowers, roots, leaves, barks and fruits of this genus could potentially produce bioactive metabolites (Conceição et al., 2023). For instance, the bioactive metabolites or secondary metabolites that exist in *Garcinia* are xanthone (Sultan et al., 2022), depsidone (Lannang et al., 2018), biflavonoid (Akongwi et al., 2023), flavonoids (Shahid et al., 2022), prenylated benzoyl phloroglucinol (Mahamodo et al., 2014), phenolic glycosides (Akongwi et al., 2023), benzoquinone (Shahid et al., 2022), isocoumarin (Nurul et al., 2018), terpene (Ji et al., 2017), and benzoate (Khamthong & Hutadilok-Towatana, 2017).

According to Nguyen et al. (2021), phenolic compounds are secondary metabolites with at least one unit of phenol in their structure. These phenolic compounds fall into various categories as outlined in Table 2.1 by Waksmundzka-Hajnos et al. (2008).

Table 2.1: Plant kingdom phenolic class

C_n basic skeleton	Condensed structure	Class of compound
6	C ₆	Simple phenols, benzoquinones, quinones
7	C ₆ -C ₁	Phenolic acids
9	C ₆ -C ₃	Hydroxycinnamic acid, coumarins
13	C ₆ -C ₁ -C ₆	Xanthones
15	C ₆ -C ₃ -C ₆	Flavonoids, isoflavonoids, anthocyanins, chalcones, aurones
30	(C ₆ -C ₃ -C ₆) ₂	Biflavonoids

2.2.1 Xanthoness

Xanthoness are ubiquitous in *Garcinia* and have been established as chemotaxonomic markers for this plant genus. Their distribution, however, is restricted to the Polygalaceae, Moraceae, Gentianaceae, Clusiaceae, and Guttiferae families (Paul & Zaman, 2022). Xanthoness or xanthene-9*H*-ones (Pedraza-Chaverri et al., 2008) are oxygenated heterocyclic compounds with a dibenzo- γ -pyrone skeleton (Paul & Zaman, 2022) that can be discovered within lichens, fungi and higher plant families (Pedraza-Chaverri et al., 2008). This secondary metabolite can be divided into xantholignoids, miscellaneous xanthoness, simple oxygenated xanthone, bixanthone, xanthone glycoside and prenylated xanthone. However, the occurrence of prenylated xanthone can be found in Clusiaceae family members only, according to Paul and Zaman (2022).

A recent study by Thuy et al. (2022) extracted the stembark of *Garcinia fagraeoides* with methanol and successfully isolated two tetraoxygenated xanthoness, which are α -mangostin (**1**) and rubraxanthone (**2**). In 2021, See et al. revealed that the chloroform extract of *G. mangostana* has contributed to the finding of three xanthoness, α -mangostin (**1**), β -mangostin (**3**) and mangaxanthone B (**4**), while methanol extracts gave one xanthone, mangostenol (**5**). Several studies also reported that a few new xanthoness were successfully isolated from another extract of *Garcinia*. For instance, the ethanol extract from the bark of *G. mangostana* yielded one novel xanthone dimer, garmoxanthone (**6**) (Wang et al., 2018). Ibrahim et al. (2019) conducted a chemical investigation on *G. mangostana* (acetone extract of pericarp) which also resulted in one new xanthone, mangostanaxanthone VIII [1,3,5,6,7-pentahydroxy-2-(3-methylbut-2-enyl)-8-(3-hydroxy-3-methylbut-1-enyl)xanth-one] (**7**). The xanthoness isolated from *G. fagraeoides* and *G. mangostana* are depicted in Figure 2.2.

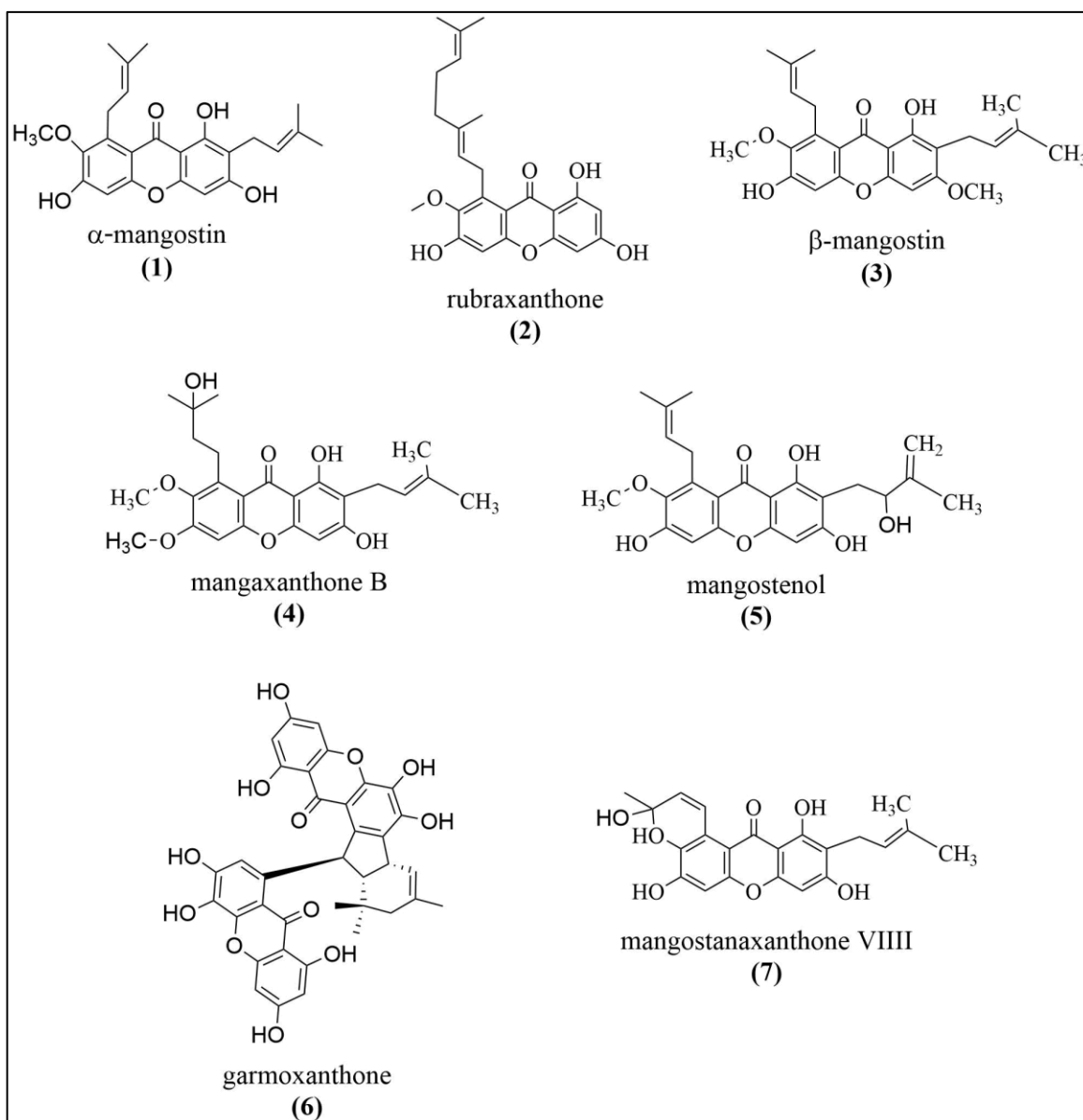


Figure 2.2: Xanthones isolated from *G. fagraeoides* and *G. mangostana*

Besides that, the investigation on the acetone extract of *G. nigrolineata* leaves led to the discovery of three new oxygenated xanthone, namely, nigrolineaxanthone X (8), nigrolineaxanthone Y (9), and nigrolineaxanthone Z (10) (Raksat et al., 2019). The work on the bark of *G. nervosa* (from chloroform extract) affords one new pyranoxanthone derivative, garner xanthone (11) (Wong et al., 2017). Another species of *Garcinia*, which is *G. cowa*, had been explored by Raksat et al. (2020) and revealed three new xanthones obtained from the acetonic extract, namely, garciniacowones J (12), garciniacowones H (13)

and garciniacowones I (**14**). Figure 2.3 shows the xanthenes isolated from *G. nigrolineata*, *G. nervosa* and *G. cowa*.

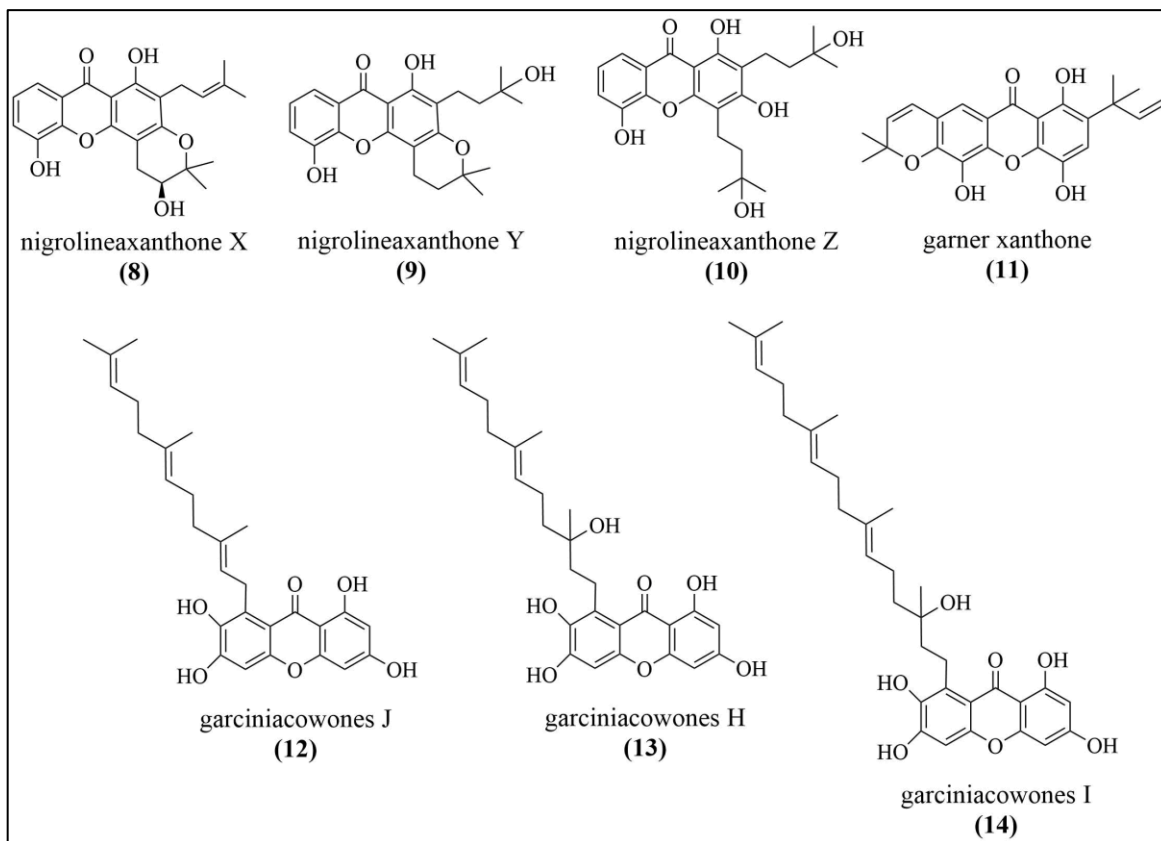


Figure 2.3: Xanthenes isolated from *G. nigrolineata*, *G. nervosa* and *G. cowa*

On the other hand, Sukandar et al. (2019) carried out a phytochemical investigation of the stem bark of *G. tetrandra* (Figure 2.4). Nine novel xanthone were successfully isolated, viz. tetrandraxanthone A (**15**), tetrandraxanthone B (**16**), tetrandraxanthone C (**17**), tetrandraxanthone D (**18**), tetrandraxanthone E (**19**), tetrandraxanthone F (**20**), tetrandraxanthone G (**21**), tetrandraxanthone H (**22**), and tetrandraxanthone I (**23**) from the methanol extract (Sukandar et al., 2019).

	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇
tetrandraxanthone A (15)		OH		H	OH	OCH ₃	
tetrandraxanthone D (18)	H	OH		H	OH	OCH ₃	
tetrandraxanthone E (19)	H	OH		H	OH	OCH ₃	
tetrandraxanthone F (20)	H	OH		H	OH	OCH ₃	

	R ₁	R ₂
tetrandraxanthone B (16)	CH ₃	
tetrandraxanthone C (17)		CH ₃

	R ₁
tetrandraxanthone A (21)	
tetrandraxanthone A (22)	
tetrandraxanthone A (23)	

Figure 2.4: Xanthenes isolated from *G. tetrandra*

2.2.2 Flavonoids

Flavonoids are produced *via* the phenylpropanoid pathway, and they comprise a large group of polyphenol compounds with a benzoyl- γ -pyrone structure that is abundant in plants (Agidew, 2022). Flavonoids can be divided into several major classes, including flavonols, isoflavones, anthocyanins, flavanones and flavones that have been proven to have a broad array of therapeutic and biological effects (Murthy et al., 2020). These flavonoids were

divided according to the degree of oxidation of the 3-C bridge (C₃-C₆-C₃) (Alseekh et al., 2020) shown in Figure 2.5.

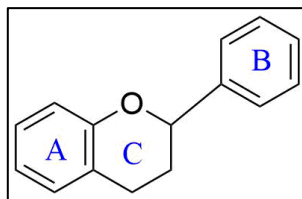


Figure 2.5: 3-C bridge (C₃C₆C₃) of flavonoids

A chemical investigation of acetone extract from leaves of *G. nigrolineata* gives two flavonoids 3',4',5,7-pentahydroxy-flavone (**24**) and 3'-deoxyquercetin (**25**) (Raksat et al., 2019). Another chemical study on the bark of *G. xanthochymus* yielded merelloflavone (**26**) (Nurul et al., 2018). The flower of *G. dulcis* also produced a few flavonoids namely rhamnazin (**27**) and quercetin 3-O-β-galactopyranoside (**28**) (Khamthong & Hutadilok-Towatana, 2017). Figure 2.6 shows the flavonoids isolated from several *Garcinia* species.

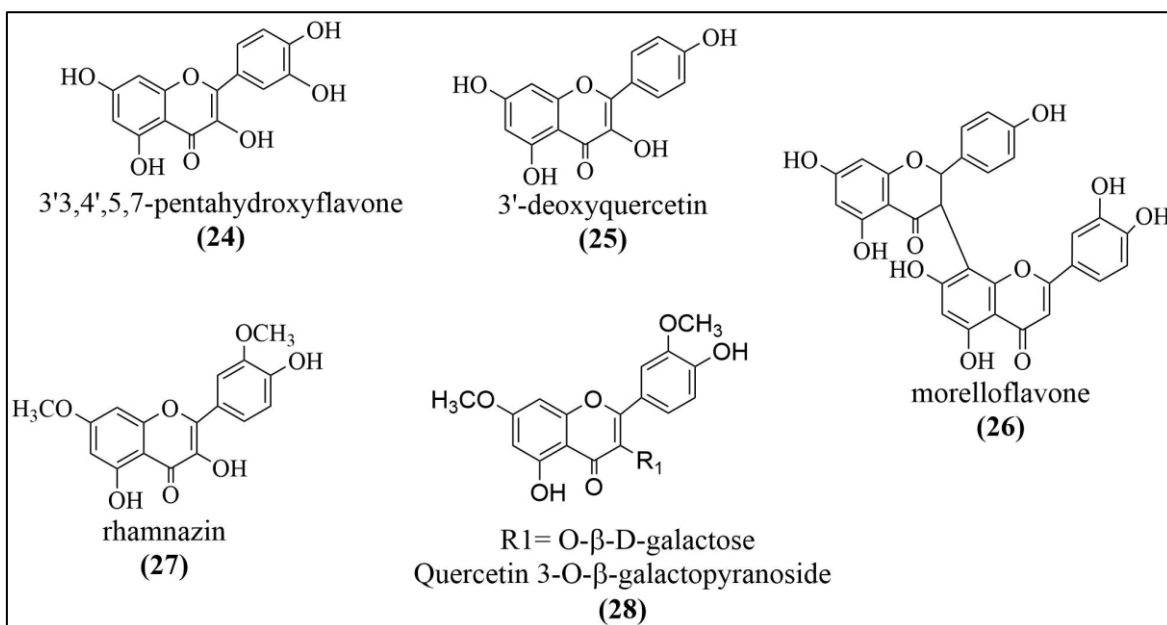


Figure 2.6: Flavonoids isolated from *Garcinia* species

2.2.3 Biflavonoids

Biflavonoids, on the other hand, are a distinct sub-class of flavonoid dimers connected by C-O-C or C-C bond and their presence is bound to *Hypericum* and *Garcinia* species only. In *Garcinia*, the biflavonoid usually consists of “two units of flavonone” or “one unit of flavone and one unit of flavonone” (Paul & Zaman, 2022). These are some of the biflavonoids compounds that have been present in *Garcinia* (Figure 2.7): brevipedicelone D (**29**) found in the leaves of *G. brevipedicellata* (Akongwi et al., 2023), amentoflavone (**30**) found in leaves of *G. morella* (Murthy et al., 2020) and *G. latissima* Miq. (Ambarwati et al., 2022), GB-2a (**31**) found in the leaves of *G. dulcis* (Khamthong & Hutadilok-Towatana, 2017), macrophylloflavone (**32**) from the methanol extract of stembark of *G. macrophylla* (Cane et al., 2020), 3'',4',4'',5,5'',7,7''-heptahydroxy-3,8''-biflavanone (**33**) and kolaflavanone (**34**) from ethanol extract from stembark *G. kola* (Akoro et al., 2020).

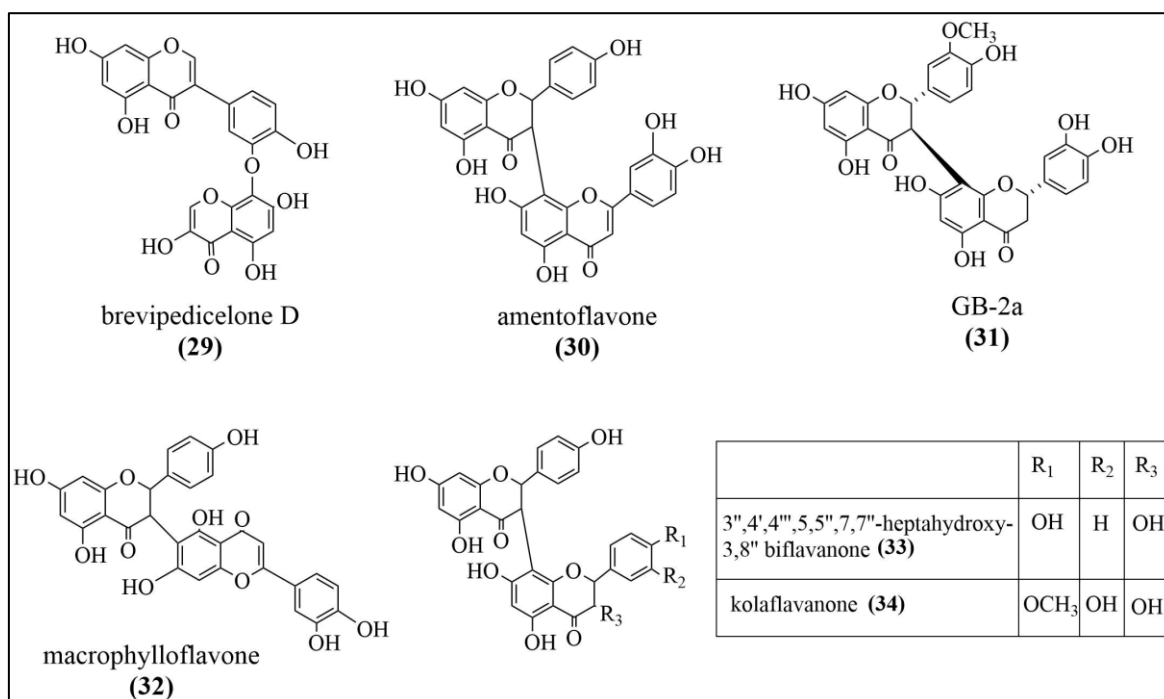


Figure 2.7: Biflavonoids isolated from *Garcinia* species

2.2.4 Other Secondary Metabolites in *Garcinia*

As previously pointed out, the genus *Garcinia* contains a large number of phenolic compounds. This includes coumarins (Naves et al., 2019), benzoquinone (Shahid et al., 2022), phloroglucinols (Paul & Zaman, 2022), benzophenones (Raksat et al., 2019) and terpenes (Delita et al., 2020).

Coumarins consist of 2*H*-1-benzopyran-2-one derivatives. It can be obtained by cyclizing C-2 oxygenated cis-cinnamic acid or through alternative pathways, such as the mixed cinnamic/acetate pathway. Moreover, coumarins can also be obtained due to a complete acetate pathway (Waksmundzka-Hajnos et al., 2008). An example of coumarin that had been reported in *Garcinia* is angelicoin B (**35**) that was found in *G. xanthochymus* shown in Figure 2.8 (Nurul et al., 2018).

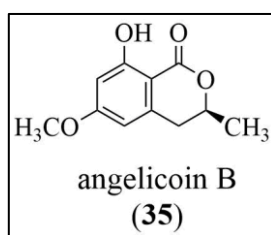


Figure 2.8: Coumarin isolated from *Garcinia* species

Conversely, benzoquinones, a class of quinones, are renowned for their wide range of biological effects. Quinones are abundant in nature and are commonly found in medicinal plants (Silva et al., 2020). Atrovirone (**36**) and 2,6-dimethoxy-p-benzoquinone (**37**) (Figure 2.9) have been reported to be present in *G. atriviridis* (Shahid et al., 2022).

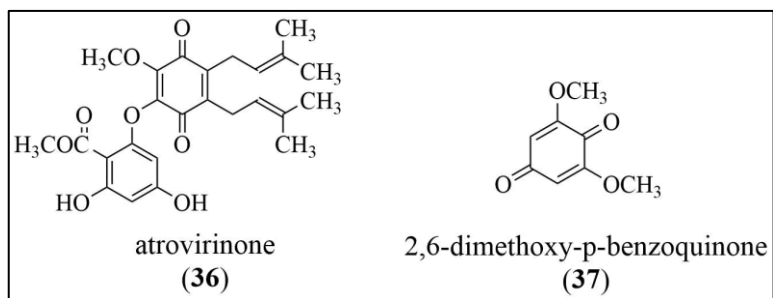


Figure 2.9: Benzoquinones isolated from *Garcinia* species

As for phloroglucinols, Paul and Zaman (2022) stated that it is a polyphenolic compound composed of three hydroxyl groups and an aromatic phenyl ring. Mahamodo et al. (2014) managed to isolate two phloroglucinol compounds from the leaves of acetone extract of *G. goudotiana*, namely, goudotianone 1 (38) and goudotianone 2 (39). Apart from the compound mentioned above, few phloroglucinol compound also had been reported to be present in the stem bark of *G. picrorhiza* which are picrorhizone H (40), picrorhizone G (41), picrorhizone F (42), picrorhizone E (43), picrorhizone D (44), picrorhizone C (45), picrorhizone B (46) and picrorhizone A (47) (Sukandar et al., 2020). Figure 2.10 displayed the phloroglucinol compound isolated from *Garcinia*.

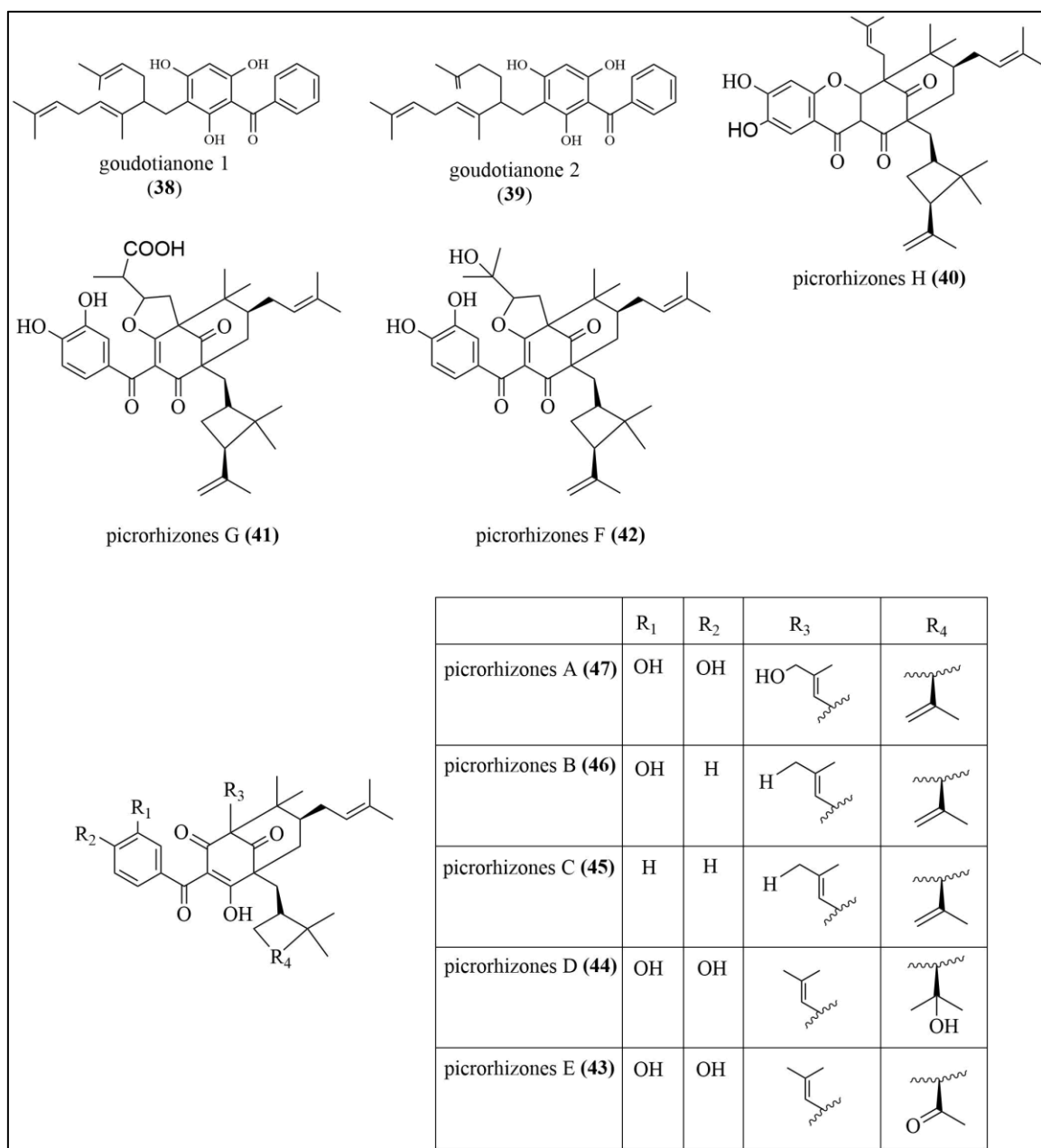


Figure 2.10: Phloroglucinols isolated from *Garcinia* species

On the contrary, *Garcinia* is a rich source of benzophenones. Raksat et al. (2019) mentioned that benzophenones are the most isolated compounds from the *Garcinia* genus. This secondary metabolite was reported to have a diverse range of biological activities. Benzophenones are produced *via* acetate-malonate and shikimic acid pathways, comprising a phenol-carbonyl-phenol skeleton (Paul & Zaman, 2022). Recently, Trinh et al. (2023) isolated two new polyisoprenylated benzophenones from ethyl acetate pericarp extract of

G. planchonii, namely, planchoniones A (**48**) and planchoniones B (**49**). In 2022, Thuy et al. extracted the stem bark of *G. fagraeoides* with methanol and successfully isolated isogarcinol (**50**). In addition, Conceição et al. (2023) reported the presence of different type of guttiferones in *Garcinia sp.* such as guttiferone A (**51**), guttiferone B (**52**), guttiferone G (**53**), guttiferone H (**54**), guttiferone I (**55**), guttiferone Q (**56**), and guttiferone E (**57**). All the benzophenones mentioned are depicted in Figure 2.11.

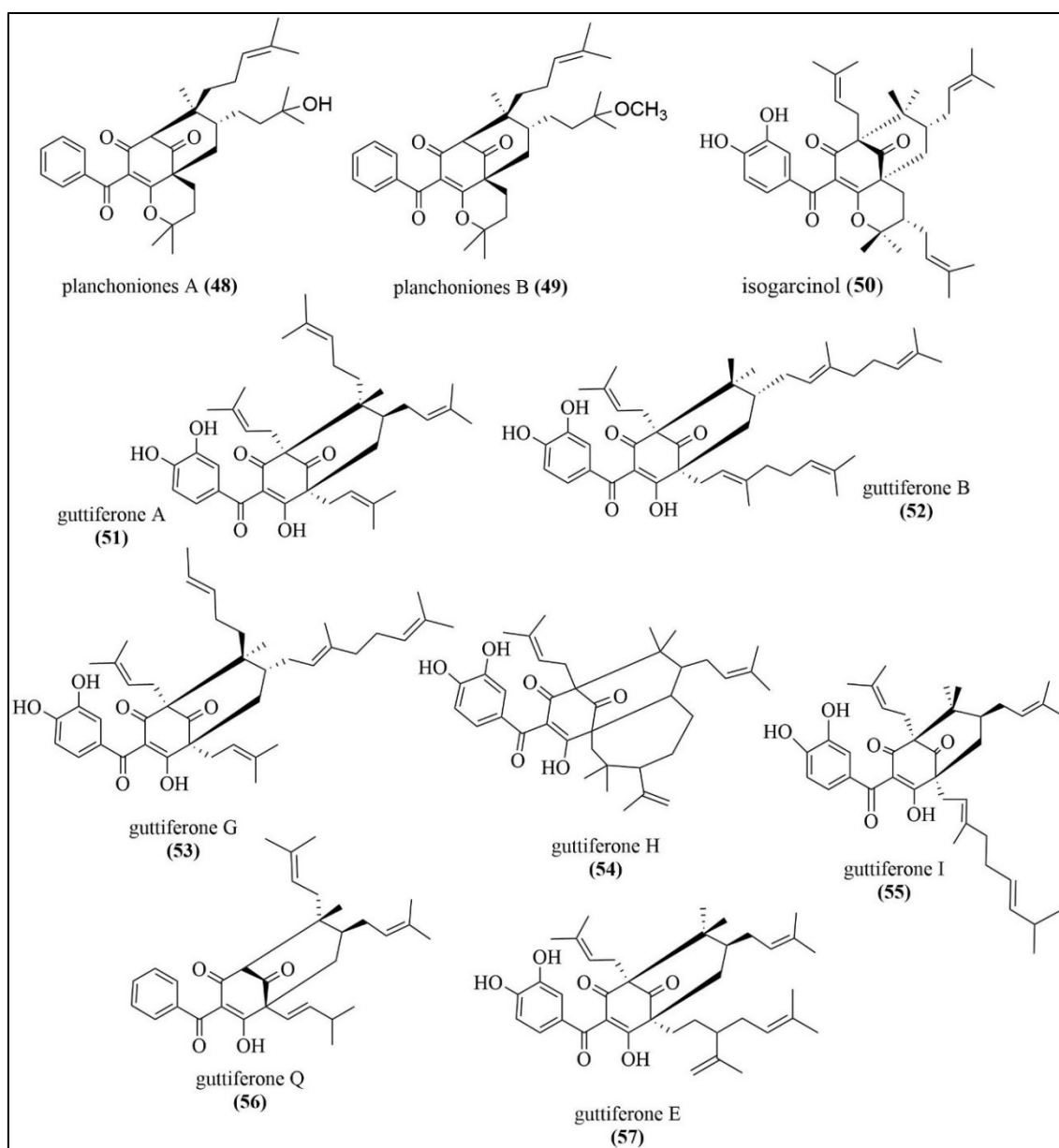


Figure 2.11: Benzophenones isolated from *Garcinia* species

Concomitantly, terpenes were also found in *Garcinia* species. Terpenes are the most diverse and most extensive natural product class identified primarily in plants (Cooper & Nicola, 2014). These are the terpenes that had been reported (Figure 2.12): β -sitosterol (**58**), stigmasterol (**59**), garcihombropane B (**60**), garcihombropane C (**61**), γ -sitosterol (**62**), (-)- β -caryophyllene (**63**) and α -humulene (**64**) (See et al., 2016; Wong et al., 2017; Muhammad et al., 2019; Delita et al., 2020; Shahid et al., 2022).

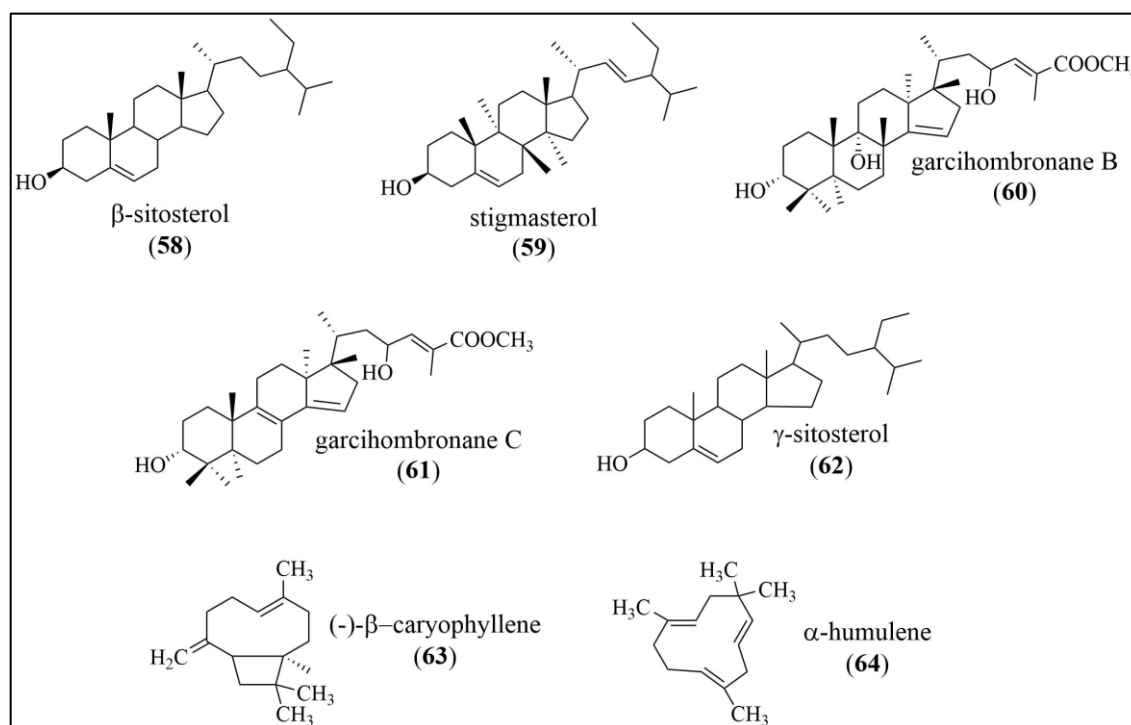


Figure 2.12: Terpenes isolated from *Garcinia* species

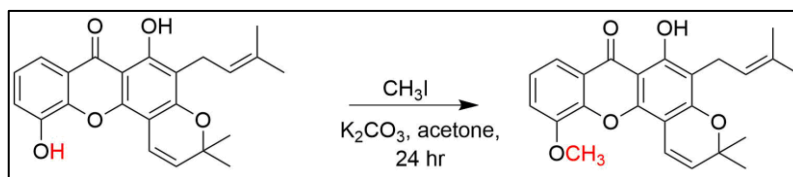
Terpenes consist of isopentane (isoprene) units with five carbon subunits derived from the isoprene rule. The metabolites can be formed through two pathways, which are the mevalonate pathway (uses Acetyl CoA as a precursor) and the non-mevalonate pathway (uses methylerythritol phosphate (MEP)) as specified by Mahizan et al. (2019). The phytochemical study of *Garcinia* withal yielded other secondary metabolites, including organic acids (Shahid et al., 2022), biphenyls (Shahid et al., 2022), benzoate

(Khamthong & Hutadilok-Towatana, 2017), and depsidone (Lannang et al., 2018; Nurul et al., 2018; Shahid et al., 2022).

2.3 Structural Modification of Xanthone

The vast biodiversity of plant species and their inherent potential for producing bioactive compounds have garnered significant scientific interest. This bioactive compound possesses intriguing biological properties, making it a prime candidate for the development of novel drugs (Huang et al., 2022). Aside from the bioactive compound itself, targeted modifications to the structures of natural resources represent a well-established approach to developing novel medications (Yao et al., 2017). By altering and refining the molecular structure, a wide array of compound libraries can be created, leveraging the potential of natural product reservoirs, and enhancing the possibilities for new drug development (Ding & Xue, 2024).

Several modified xanthenes have been reported that can enhance biological activity. For instance, structural changes through esterification on ananixanthone (**65**) (Scheme 2.1) increased cytotoxic activity compared to the parent compound (Lee et al., 2018). Aside from that, Koh et al. (2015) and Zou et al. (2013) reported increased anti-bacterial activity upon modification of α -mangostin (**1**). Conversely, Karunakaran et al. (2018) observed cytotoxicity and anti-inflammatory properties from the structural modification of β -mangostin (**3**). These studies revealed that modifications to natural compounds have the potential to enhance their biological activities. It also demonstrated the potential of modified xanthenes as a novel therapeutic agent.



Scheme 2.1: Structural modification of ananixanthone

2.4 Anti-microbial Activity of *Garcinia* Genus

Nowadays, the rise of antibiotic-resistant bacteria is a major source of concern. Antibiotic misuse contributes to rising bacterial resistance, as indicated by the Centre for Disease Control and Prevention (Joana et al., 2019). A study by Elmaidomy et al. (2022) found that the most resistant bacteria to antibiotics are *Acinetobacter baumannii*, *Enterobacter sp.*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* for Gram-negative bacteria and *Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* for Gram-positive bacteria. These bacteria are resistant to a wide range of antibiotics, making them difficult to treat. Another study by Piechota et al. (2018) revealed that one of the most serious problems is the *Methicillin-Resistant Staphylococcus aureus* strains (MRSA). MRSA is a pathogen that rapidly evolving and has become one of the most common sources of infections that are resistant to multiple drugs, particularly in developing countries (Kuok et al., 2017). Despite alternative drugs (vancomycin and quinolones) that have been identified as MRSA treatments, their efficacy is still decreasing (Gorlenko et al., 2020). As a result, it is critical to develop new anti-microbial agents using various strategies to minimize mutations or other resistance mechanisms (Joana et al., 2019).

A large number of secondary metabolites from plants have shown promising activity against bacteria, making them a potential source of new anti-bacterial (Akongwi et al., 2023). Gorlenko et al. (2020) stated that numerous research investigations have found that

secondary metabolites from plants have potent anti-microbial activity against *S. aureus* strains, including MRSA. The properties and chemical structure of these metabolites determine their mode of action. An example can be seen by the presence of hydroxyl (-OH) and methoxy (-OCH₃) groups in the chemical structure of plant-derived compounds significantly affects their antimicrobial potential. These groups allow the compounds to diffuse through bacterial membranes, resulting in cytoplasmic acidification and, ultimately, cell death (Alibi et al., 2021). Besides, secondary metabolites can have a variety of effects on the cells of microbes. For instance, interrupting the quorum sensing, induction of coagulation of cytoplasmic constituents, preventing the synthesis of enzymes, destabilization of the proton motive force with ion leakage, interfering with DNA/RNA function and synthesis, interacting with membrane proteins (ATPases and others), as well as disrupting the structure and function of the cytoplasmic membrane (Gorlenko et al., 2020).

Nguyen et al. (2021) reported that *Garcinia* is an important anti-microbial activity source. Prior research has revealed that the crude extract from the *Garcinia* species has anti-microbial properties (Khamthong & Hutadilok-Towatana, 2017; Akongwi et al., 2023). This genus also contains numerous secondary metabolites with a diverse set of biological and pharmacological properties, including anti-microbial properties (Ji et al., 2017). A similar study has been reported by Gorlenko et al. (2020), where the researcher revealed that secondary metabolites of plants possessed anti-microbial activity, and the metabolites extracted were found to have bacteriostatic or bactericidal activity against microorganisms. Metabolites that are bacteriostatic can inhibit bacterial cellular growth and activity. Meanwhile, metabolites that are bactericidal can kill bacteria by inhibiting the function of the cell membranes, the synthesis of the cell wall, or the synthesis of enzymes or proteins (Ecevit et al., 2022).

Various approaches have been used to assess anti-bacterial activity, including the diffusion and dilution methods. Diffusion methods consist of disc diffusion, agar well diffusion method, agar plug diffusion method or cross streak method usually used to test the anti-microbial potency of pure substances, extracts, or fractions (Balouiri et al., 2016). The anti-microbial activity can be evaluated by measuring the diffusion method's diameter of the inhibition zone (IZD) (Zamakshshari et al., 2022). Conversely, the dilution assay is usually used to determine the minimum inhibitory concentrations (MIC) and the minimum bactericidal concentration (MBC). The relevance of anti-microbial properties can be classified into two parts which are the extract and the secondary metabolites. Porras et al. (2021) reported that the secondary metabolites possess significant anti-bacterial activity at MICs ≤ 100 $\mu\text{g/mL}$, where MIC range 11 to 100 $\mu\text{g/mL}$ indicates moderate activity and MIC ≤ 10 $\mu\text{g/mL}$ indicates active activity. Conversely, the extract is considered active when MICs ≤ 100 $\mu\text{g/mL}$, moderate (100 $\mu\text{g/mL} < \text{MIC} < 500$ $\mu\text{g/mL}$), weak (500 $\mu\text{g/mL} < \text{MIC} < 1000$ $\mu\text{g/mL}$), and inactive when MIC > 1000 $\mu\text{g/mL}$ (Naves et al., 2019).

2.4.1 Anti-microbial Activity of *Garcinia* Crude Extract

Crude extracts contain multiple secondary metabolites that can act at different sites in microbial cells, resulting in an overall anti-microbial activity (Gorlenko et al., 2020). In the 1990s, Iinuma and his colleagues discovered that extracts from the mangosteen fruit (*G. mangostana*) have potential anti-bacterial properties against MRSA (Sultan et al., 2022). Subsequently, numerous studies have demonstrated the anti-microbial activity of *Garcinia* species, whereby some researchers using different types of extracts (petroleum ether, hexane, dichloromethane (DCM), chloroform (CHCl_3), ethyl acetate (EA), acetone, methanol (MeOH), ethanol (EtOH), aqueous (Aq)) and different part of *Garcinia* plant (seeds, fruits, fruit rinds, stembark, pericarp, leaves, roots, branch) in their study.

Paul and Zaman (2022) reviewed several *Garcinia* species where some of the extract had anti-bacterial activity. For instance, the petroleum ether crude extract (from *G. xanthochymus*'s seed), chloroform crude extract (from *G. dulcis*'s seed and *G. mangostana*'s fruit rind), acetone extract (from *G. dulcis*'s stembark, fruits of *G. cowa*, latex of *G. morella*), ethanol extract (from fruit rinds of *G. mangostana*) and aqueous crude extract (from fruit *G. morella*). Aside from that, Murmu et al. (2016) reported that different solvent extracts of the fruits of *G. xanthochymus* (hexane, petroleum ether, acetone, and aqueous extract) showed anti-bacterial activity against *S. mutans*, *S. pyogens*, *Shigella flexneri*, *Salmonella typhimurium*, and *Vibrio cholerae*. The control (Gatifloxacin, Gentamycin, Kanamycin and Neomycin) had better inhibitory effects than the extracts, but the extracts still showed a significant zone of inhibition (Murmu et al., 2016). In addition, Khamthong and Hutadilok-Towatana (2017) reveal that the hexane extract of *G. dulcis*'s stembark is potent against *S. pneumoniae*, MRSA, and *S. aureus*.

In a previous study, See et al. (2016) conducted an *in vitro* anti-bacterial assay using different solvent extracts of the stembark of *G. benthamiana* (ethyl acetate, chloroform and hexane). The researchers found that all extracts had minimal inhibitory activities against *S. aureus* (IZD: 7 mm) and *Bacillus subtilis* (IZD: range 7-8 mm), when compared to the positive control (Streptomycin: IZD: 26-29 mm). On the other hand, in 2017, Janardhanan et al., also had conducted an *in vitro* study on anti-microbial activity from *G. mangostana*. The diffusion result reveal that the chloroform extract from *G. mangostana* pericarp has activity towards all tested bacteria strains with low activity against *S. salivarius* (IZD: 3 mm), moderate activity against *S. mutans* (IZD: 10.6 mm) and *S. oralis* (IZD: 11.3 mm), and highest activity against *S. sanguinis* (IZD: 13.6 mm) and *Lactobacillus acidophilus* (IZD: 13.6). As for the dilution method, *L. acidophilus* (MIC 25 mg/mL, MBC 50 mg/mL) was

nearly equivalent to the positive control (MIC 20 mg/mL), whereas the MIC and MBC for other bacterial strains (*S. salivarius*, *S. mutants*, *S. oralis*, *S. sanguinis*) are in the range MIC 50-100 mg/mL and MBC 100-200 mg/mL, respectively (Janardhanan et al., 2017).

According to Perry et al. (2022), bactericidal secondary metabolites can potentially promote the development of microbial drug resistance. This is due to harnessing plant metabolites is a cost-effective strategy for developing novel antimicrobials or improving existing antibiotics to combat resistance (Keita et al., 2022).

In recent study by Murthy et al. (2020), the researcher reviewed an anti-microbial study using methanol extract of *G. morella*. Interestingly, *G. morella* methanolic extract showed exceptional anti-bacterial activity against *S. aureus* and *B. cereus*, with MIC values of 500 and 200 µg/mL, respectively. In 2021, Nguyen and colleagues reported that the methanolic extract of *G. planchonii* arrested the growth of both Gram-positive (*B. subtilis* (IZD: 16 ± 1.2 mm) and *S. aureus* (IZD: 17 ± 2.5 mm)) and Gram-negative (*P. aeruginosa* (IZD: 15 ± 1.5 mm) and *E. coli* (IZD: 20 ± 1.2 mm)) bacterial strain. Meanwhile, *G. nigrolineata* and *G. tinctoria* show inhibition zone towards *P. aeruginosa* (IZD: 9 ± 1.4 mm) and *S. aureus* (IZD: 10 ± 0.9 mm), respectively. Akongwi et al. (2023), on the other hand, had prepared ethyl acetate extracts of *G. epunctata* as well as ethyl acetate, acetone, and methanol extracts of *G. brevipedicellata* to test their anti-bacterial activity against *Multidrug-Resistant Salmonella*. The results reveal that low minimum bactericidal concentrations (≤ 2 mg/mL) were recorded against most bacteria strains which may indicate bactericidal activity.

Several other studies by Tanha (2017) Larsuprom et al. (2019), Musa et al. (2020), and Devi et al. (2019) have demonstrated the anti-microbial properties of extracts towards

Gram-positive and Gram-negative bacteria using the dilution method. The extracts primarily target Gram-positive rather than Gram-negative strains. Gram-positive bacteria are more susceptible to plant extracts from the Clusiaceae family due to a few factors including differences in peptidoglycan structure, the presence of an outer membrane (Yan et al., 2021), receptors or lipids, cross-linking (Breijyeh et al., 2020), and the activity of the autolytic enzymes that control the compounds' penetration, binding, and action (Nguyen et al., 2021). However, some of the extracts were found to have low activity. This might be due to interference with the metabolites of other extract components (Nguyen et al., 2021). Overall, most of the *Garcinia* crude extract does show promising anti-bacterial activity.

2.4.2 Anti-microbial Activity of *Garcinia* Secondary Metabolites

Natural-based compounds are a top-interest candidate in combating microbial infections and preventing the emergence of drug-resistant strains because they can interact with the microbial cell through multiple anti-microbial mechanisms (Ecevit et al., 2022). Natural secondary metabolites with a molecular weight of ≤ 500 g/mol may be able to act as anti-microbial adjuvants and exhibit synergistic effects (Mahizan et al., 2019). Apart from that, Gorlenko et al. (2020) stated that secondary metabolites with high bactericidal activity can stimulate the development of microbial drug resistance. It has been claimed that *Garcinia* contains a wide range of biologically active secondary metabolites including the anti-microbial secondary metabolites.

Natural phenolic compounds are a diverse group of compounds with a wide range of biological activities, including anti-bacterial (Ecevit et al., 2022). Few studies have reported the presence of the phenolic compound in the genus of *Garcinia* (Figure 2.13). Phloroglucinol, for instance, a simple phenol, is rarely found alone in plants (Ecevit et al.,

2022). It is usually found in combination with either benzoic acid or cinnamic acids. Goudotianone 1 (**38**) and goudotianone 2 (**39**), prenylated benzoyl phloroglucinol derivatives, were isolated by Mahamodo et al. (2014) from the acetone crude extract of *G. goudotiana* through bioassay-guided fractionation. These compounds were very active against Gram-positive bacteria (**38** active against *E. faecalis* and **39** active against *S. lugdunensis*). Another example from phenolic compounds, which are phenolic glycosides, named epunctosides A (**66**) and epunctosides B (**67**) from *G. epunctata* have low activity against MDRS clinical isolates (Akongwi et al., 2023). Benzyl 2,4-dihydroxy-6-propylbenzoate (**68**) (Khamthong & Hutadilok-Towatana, 2017), a benzoate, as well as benzophenone, namely 7-epiclusianone (**69**), garcinol (**70**), xanthochymol (**71**) (Santo et al., 2020), camboginol (**72**), isogarcinol (**50**) (Paul & Zaman, 2022), guttiferone I (**55**), isoxanthochymol (**73**) and guttiferone A (**51**) (Conceição et al., 2023) founded in *Garcinia* species were revealed to have anti-microbial activity towards Gram-positive and/or Gram-negative bacteria strain.

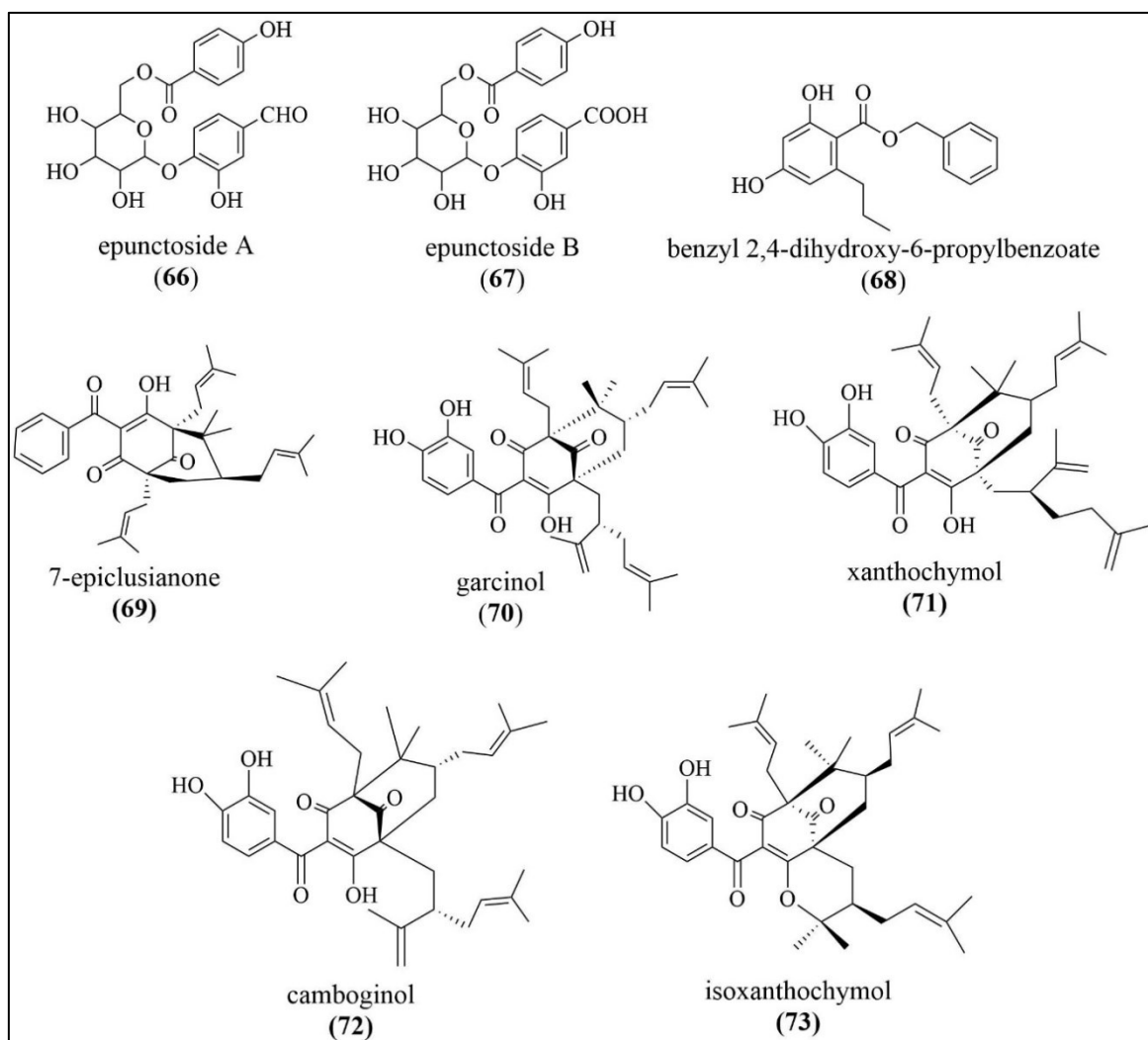


Figure 2.13: Phenolic compounds possessed anti-bacterial activity

Conversely, benzoquinones, a class of quinones, are particularly noteworthy for their biological effects (Silva et al., 2020). Quinones are well-known molecules derived from medicinal plants that are abundant in nature. Intriguingly, Junior et al. (2022) stated that the anti-bacterial properties of quinone derivatives have led to further investigation for potential use in a variety of clinical and pharmaceutical applications. The hydrogen's presence in the quinone structure renders it more reactive in combating microorganisms. This is due to hydrogen atoms in the quinone structure allows for hydrogen bonding with various biological molecules, enhancing their binding affinity to microbial targets (Badave et al., 2020). In addition, quinones ability to develop new drugs is enhanced by the presence of an

electron-rich aromatic ring that can act in electrophilic substitution (Junior et al., 2022). An example of anti-microbial activity has been reported for benzoquinone in *Garcinia*, 2,6-dimethoxy-p-benzoquinone (**37**), and atrovirinone (**36**) from *G. atroviridis* (Shahid et al., 2022).

On the other hand, flavonoids are rich and engage in various roles (Cooper & Nicola, 2014), including possessing anti-microbial activity. According to Gorlenko et al. (2020), flavonoids' anti-microbial activity is due to their interaction with membrane proteins on bacterial cell walls. This interaction increases the membrane's permeability, disrupting it and eventually killing the bacteria. Aside from that, Cane et al. (2020) reported that flavonoids' anti-bacterial properties can be assessed based on their capacity to obstruct nucleic acid synthesis, cytoplasmic membrane function, energy metabolism, and porins in cell membranes. Besides flavonoids, many biflavonoids, particularly those derived from the *Garcinia* genus, also have anti-bacterial properties (Fotso et al., 2014). Prior research teams had certified flavonoid as well as biflavonoid compounds such as compounds **24**, **25**, **27**, **29**, **30**, **31** and **32** have exhibited anti-bacterial activity (Khamthong & Hutadilok-Towatana, 2017; Raksat et al., 2019; Cane et al., 2020; Ambarwati et al., 2022; Akongwi et al., 2023).

A series of experiments also revealed numerous xanthones for anti-bacterial activity. The derivative of xanthone can have a variety of activities, including anti-microbial activity as reported by Joana et al. (2019). α -mangostin (**1**), one of nature's most researched xanthones (Joana et al., 2019), has been shown to have anti-microbial activity against a wide range of microbes, including bacteria, fungi, and mycobacteria (Sultan et al., 2022). Compound **1** anti-microbial action is due to its ability to depolarize the bacterial membrane, causing the leakage of approximately 37% of its contents within a very short period

(Sultan et al., 2022). Aside from that, cowanin (**74**), also had been discovered to be the most potent inhibitor due to the presence of double bonds in the geranyl substituent at C-8, which proved to be an important factor in the anti-bacterial activity of xanthone with a 1,3,7-trihydroxy-7-methoxy oxidation pattern (Muhammad et al., 2019).

Muhammad et al. (2019) stated that the anti-bacterial activity of xanthenes varies depending on the inclusion and position of geranyl or prenyl substituents as well as hydroxyl groups. The hydroxyl groups, especially at C-6 and C-3, are particularly important in inhibiting bacterial activity. Meanwhile, the prenyl side chain at C-2 also enhances anti-bacterial activity.

Besides prenylated benzoyl phloroglucinol, phenolic glycosides, benzoate, benzophenone, benzoquinone, flavonoid, biflavonoid and xanthone, depside (atrovirisidone (**75**)) that were found in *Garcinia* species also possess anti-bacterial activity (Shahid et al., 2022). Concomitantly, terpenes were also present in *Garcinia* species. Terpenes and terpenoids have anti-microbial properties due to their ability to interact with and damage the membranes of microbes (Gorlenko et al., 2020). Terpenoids are derived from terpenes and are formed when terpenes are modified, such as by adding or removing functional groups (Mahizan et al., 2019). Their functional group determines the anti-microbial activity of terpenoids. Aside from that, the phenolic terpenoids' delocalized electron and hydroxyl group are among the anti-microbial determining factors (Mahizan et al., 2019). Some examples of terpenes that had claimed to have anti-bacterial properties against the tested bacterial strains were (–)- β -Caryophyllene (**63**), α -humulene (**64**), β -sitosterol (**58**) and stigmasterol (**59**).

Extensive research on plant phytochemicals for drug discovery have been conducted. This includes discovering the *Garcinia* species which intensively reported due to its potential for diverse medicinal properties, including anti-microbial. The genus *Garcinia* has been linked to an abundance of secondary metabolites (Wong et al., 2017). The anti-microbial activity of *Garcinia* secondary metabolites has been the subject of much experimental and theoretical research.

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Plant Materials

The stem bark of *Garcinia dryobalanoides* (Voucher Specimen no. UiTM3032) was collected from Semenggoh, Sarawak, and was deposited at the UiTM herbarium. The sample was identified by a botanist.

3.2 Chemicals and Reagents

3.2.1 Extraction

Three different solvents were used for the plant extraction: hexane (C_6H_{14}), ethyl acetate ($C_4H_8O_2$), and methanol (CH_3OH). All the chemicals and reagents were of American Chemical Society (ACS) or Analytical Reagent (AR) grade purchased from local supplier such as Serba Teknik Saintifik Sdn. Bhd., Robert Scientific Company Sdn. Bhd., and Modern Scientific Sdn. Bhd.

3.2.2 Isolation and Purification

Five types of different solvents were used for the isolation and purification of secondary metabolites: hexane (C_6H_{14}), chloroform ($CHCl_3$), ethyl acetate ($C_4H_8O_2$), acetone (C_3H_6O) and methanol (MeOH). Aside from that, the silica gel in different sizes such as Silica gel Merck Kieselgel 60 Art. No. 7734.100 of particle size 0.063 to 0.200 mm, Silica gel Merck Kieselgel 60 Art. No. 9385.100 of particle size 0.040 to 0.063 mm and Sephadex LH-20 were used in column chromatography. All the chemicals and reagents were of American Chemical Society (ACS) grade.

3.2.3 Instrumental Analysis

Deuterated solvents (chloroform, acetone, methanol) were used for NMR, whereas HPLC/ LC-MS grade solvent was used for instrumental analysis.

3.2.4 Anti-bacterial Assay

Four bacterial strains comprising one Gram-positive (*Lactiplantibacillus plantarum*) and three Gram-negative (*Enterobacter cloacae*, *Pseudomonas aeruginosa* and *Serratia marcescens*) were used for this work. Ampicillin was used as the positive control, while dimethyl sulfoxide (DMSO) was used as the negative control.

3.3 Extraction, Isolation and Purification of Secondary Metabolites

3.3.1 Extraction, Isolation and Purification

The stembark of *G. dryobalanoides* was dried and ground into a fine powder before being macerated three times sequentially in three different solvents of different polarities, beginning with hexane (C₆H₁₄), followed by ethyl acetate (C₄H₈O₂), and lastly, methanol (CH₃OH) for 72 hours at room temperature. The sample were filtered and evaporated using a rotary evaporator to obtain the extracts. The sample extract was further purified using the chromatographic method. Each extract was fractionated using Silica gel Merck Kiesel gel 60 Art. No. 7734.100 column chromatography. Then, each fraction underwent extensive purification using column chromatography using Silica gel Merck Kiesel gel 60 Art. No. 9385.100 and using Sephadex LH-20. The sample's extraction, isolation and purification were prepared following the literature procedure by See et al. (2021).

3.3.2 Chromatographic Method

Chromatographic techniques have been effectively used to fractionate and purify biological secondary metabolites from various samples (Bajpai et al., 2016). Thus, this study used two chromatographic methods: column chromatography (CC) and thin layer chromatography (TLC).

3.3.2.1 Column Chromatography (CC)

Column chromatography (glass column packed with silica gel) was used to separate the compound mixture. Two types of silica gel were used for column chromatography: Merck Kiesel gel 60 Art. No. 9385.100 of particle size 0.040-0.063 mm, where the compound mixture was separated based on the polarities and Sephadex LH-20, where the compound mixture was separated based on the molecular size.

3.3.2.2 Thin Layer Chromatography (TLC)

The compound purity was determined by using analytical thin layer chromatography (TLC) before proceeding to other analyses such as NMR, LC-MS, FT-IR, UV-vis and melting point. In this study, TLC Silica gel 60 F₂₅₄ aluminium sheets 5 × 5 cm and different solvent system for its mobile phase (C₆H₁₄: CHCl₃ ; C₆H₁₄: EA ; C₆H₁₄: C₃H₆O ; CHCl₃: C₃H₆O ; CHCl₃; MeOH) was used.

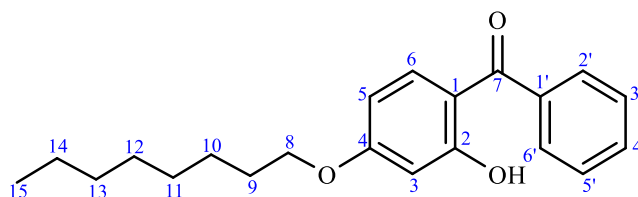
3.4 Structural Elucidation and Characterization

The isolated compounds' structures were elucidated using spectroscopic analysis, which includes Nuclear Magnetic Resonance (1D and 2D-NMR), Liquid Chromatography Mass- Spectrometry (LC-MS), UV-Visible Spectrophotometer (UV-vis) and Fourier Transform Infrared Spectroscopy (FT-IR). The 1D and 2D NMR were generated using JOEL

JNM-ECZ 500R (500 MHz) and BRUKER 500 spectrometer (400 MHz). The deuterated solvents such as chloroform, acetone, and methanol were used for NMR analysis. ATR on THERMO scientific NICOLET iS10 FT-IR spectrometer was used to obtain the infrared spectra for this study. LC-MS spectra were obtained using a Shimadzu LCMS-2020 spectrometer. For UV-vis, the spectra were determined using an Agilent Cary 60 UV-Vis spectrometer, where the spectra were recorded in a specified range. The sample was measured using Stuart, SMP3 Melting Point Apparatus for melting points.

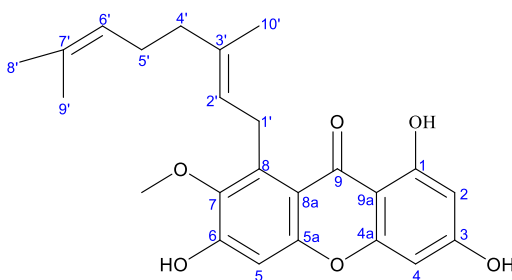
3.5 Physical and Spectral Data of Secondary Metabolites from *Garcinia dryobalanoides*

3.5.1 2-Hydroxy-(4-octyloxy)benzophenone (76)



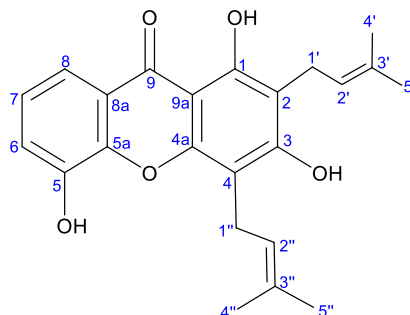
Light yellow gum; LC-MS (m/z): 326.45; $C_{21}H_{26}O_3$; UV-vis ($\lambda_{\max}$): 285; IR $\nu_{\max}$ cm^{-1} : 2923.72, 1618.09, 1255.52; ^1H NMR (CD_3OD , 400 MHz) δ_{H} : 7.48 (m , 1H, H-6), 7.62 (m , 2H, H-2', H-6'), 7.53 (m , 2H, H-3', H-5'), 7.61 (m , 1H, H-4'), 6.50 (d , 1H, $J=2.4$, H-3), 6.45 (dd , 1H, $J=2.4, 9.0$, H-5), 4.04 (t , 2H, $J=6.4, 6.5$, H-8), 1.79 (m , 2H, H-9), 1.48 (m , 2H, H-10), 1.31-1.36 (m , 8H, H-11—H-14), 0.93 (t , 3H, $J=6.9, 7.0$, H-15); ^{13}C NMR (CD_3OD , 100 MHz) δ_{C} : 112.8 (C-1), 165.8 (C-2), 101.3 (C-3), 166.0 (C-4), 107.2 (C-5), 135.1 (C-6), 200.1 (C-7), 68.2 (C-8), 28.7 (C-9), 25.7 (C-10), 28.9 (C-11), 29.0 (C-12), 31.6 (C-13), 22.3 (C-14), 13.1 (C-15), 138.3 (C-1'), 128.5 (C-2'), 128.0 (C-3'), 131.3 (C-4'), 128.0 (C-5'), 128.5 (C-6'). ^1H and ^{13}C NMR spectral data are consistent with Khamidullina et al. (2005).

3.5.2 Rubraxanthone (2)



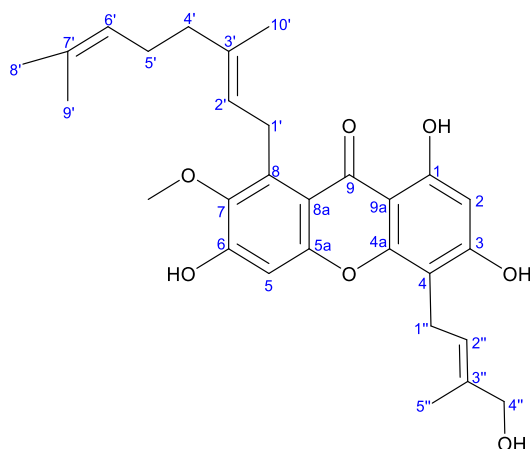
Orange crystal; m.p. 205 °C (Lit 207-208 °C) (Thuy et al., 2022); LC-MS (m/z): 410.45; C₂₄H₂₄O₆; UV-vis (λ_{max}): 240, 310; IR ν_{max} cm⁻¹: 3421.31, 2911.68, 1642.94, 1463.36, 1074.44; ¹H NMR (acetone-d₆, 400 MHz) δ_{H} : 13.47 (*s*, 1H, 1-OH), 6.15 (*d*, 1H, *J*=2.2, H-2), 6.23 (*d*, 1H, *J*=2.2, H-4), 6.73 (*s*, 1H, H-5), 3.79 (*s*, 3H, 7-O-CH₃), 4.07 (*d*, 2H, *J*=6.4, H-1'), 5.27 (*t*, 1H, *J*=5.6, 6.4, H-2'), 1.97 (*m*, 2H, H-4'), 2.02 (*m*, 2H, H-5'), 5.00 (*t*, 1H, *J*=6.6, 6.8, H-6'), 1.53 (*s*, 3H, H-8'), 1.49 (*s*, 3H, H-9'), 1.80 (*s*, 3H, H-10'); ¹³C NMR (acetone-d₆, 100 MHz) δ_{C} : 163.9 (C-1), 97.8 (C-2), 164.3 (C-3), 92.9 (C-4), 156.9 (C-4a), 101.9 (C-5), 155.3 (C-5a), 156.4 (C-6), 143.6 (C-7), 60.5 (7-O-CH₃), 137.2 (C-8), 111.1 (C-8a), 181.7 (C-9), 102.9 (C-9a), 25.9 (C-1'), 124.0 (C-2'), 134.1 (C-3'), 39.6 (C-4'), 26.4 (C-5'), 124.3 (C-6'), 130.6 (C-7'), 24.9 (C-8'), 16.9 (C-9'), 15.8 (C-10'). The ¹H and ¹³C NMR spectral data are comparable with Thuy et al. (2022).

3.5.3 8-Deoxygartanin (77)



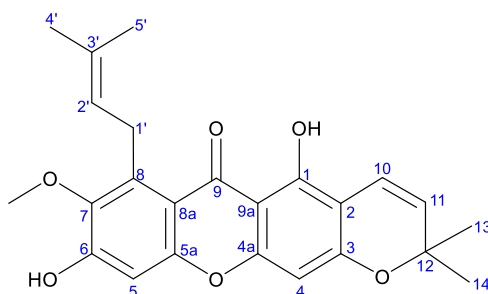
Yellow amorphous powder; m.p. 167 °C (Lit 165-167 °C) (Karunakaran et al., 2022); LC-MS (m/z): 380.45; C₂₃H₂₄O₅; UV-vis (λ_{max}): 265, 315, 385; IR ν_{max} cm⁻¹: 3269.39, 2916.54, 1638.22, 1476.36, 1093.33; ¹H NMR (acetone-d₆, 400 MHz) δ_{H} : 13.29 (s, 1H, 1-OH), 7.37 (dd, 1H, *J*=3.0, 9.0, H-6), 7.48 (d, 1H, *J*=9.0, H-7), 7.59 (d, 1H, *J*=3.0, H-8), 3.45 (d, 2H, *J*=6.9, H-1'), 5.25 (t, 2H, *J*=7.0, 7.1, H-2'/2''), 1.80 (s, 3H, H-4'), 1.67 (s, 6H, H-5'/5''), 3.59 (d, 2H, *J*=7.1, H-1''), 1.89 (s, 3H, H-4''); ¹³C NMR (acetone-d₆, 100 MHz) δ_{C} : 159.7 (C-1), 111.3 (C-2), 161.8 (C-3), 107.2 (C-4), 154.4 (C-4a), 151.2 (C-5), 155.1 (C-5a), 125.4 (C-6), 120.2 (C-7), 109.7 (C-8), 122.0 (C-8a), 182.0 (C-9), 104.0 (C-9a), 22.6 (C-1'), 123.5 (C-2'/2''), 132.9 (C-3'), 18.4 (C-4'), 26.3 (C-5'/5''), 22.9 (C-1''), 132.7 (C-3''), 18.5 (C-4''). The ¹H and ¹³C NMR spectral data are in consistent with Nguyen et al. (2003).

3.5.4 Isocowanol (78)



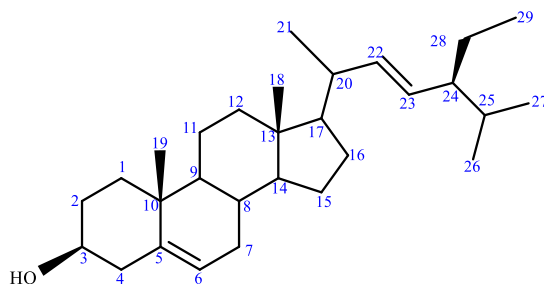
Orange gum; LC-MS (m/z): 494.60; $C_{29}H_{34}O_7$; UV-*vis* ($\lambda_{\max}$): 225, 260, 315; IR $\nu_{\max}$ cm^{-1} : 3410.91, 2927.96, 1644.50, 1463.27, 1072.25; ^1H NMR ($CDCl_3$, 400 MHz) δ_H : 13.40 (*s*, 1H, 1-OH), 6.26 (*s*, 1H, H-2), 6.87 (*s*, 1H, H-5), 3.80 (*s*, 3H, 7-O-CH₃), 4.08 (*d*, 2H, $J=5.9$, H-1'), 5.26 (*t*, 1H, $J=5.8, 6.0$, H-2'), 2.02 (*m*, 2H, H-4'), 2.04 (*m*, 2H, H-5'), 5.02 (*t*, 1H, $J=6.3, 6.4$, H-6'), 1.54 (*s*, 3H, H-8'), 1.59 (*s*, 3H, H-9'), 1.82 (*s*, 3H, H-10'), 3.57 (*d*, 2H, $J=7.8$, H-1''), 5.42 (*t*, 1H, $J=7.8, 7.9$, H-2''), 4.39 (*s*, 2H, H-4''), 1.78 (*s*, 3H, H-5''); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C : 161.6 (C-1), 98.8 (C-2), 161.5 (C-3), 103.4 (C-4), 153.9 (C-4a), 101.6 (C-5), 154.7 (C-5a), 155.7 (C-6), 142.8 (C-7), 62.0 (7-O-CH₃), 137.2 (C-8), 111.9 (C-8a), 182.1 (C-9), 103.7 (C-9a), 26.5 (C-1'), 123.2 (C-2'), 135.6 (C-3'), 39.7 (C-4'), 26.6 (C-5'), 124.3 (C-6'), 131.3 (C-7'), 17.7 (C-8'), 25.6 (C-9'), 16.5 (C-10'), 21.7 (C-1''), 126.7 (C-2''), 133.5 (C-3''), 62.7 (C-4''), 22.7 (C-5''). The ^1H and ^{13}C NMR spectral data agree with Taher et al. (2007).

3.5.5 9-Hydroxycalabaxanthone (79)



Orange oil; LC-MS (m/z): 408.45; $C_{24}H_{24}O_6$; UV- vis (λ_{max}): 285; IR ν_{max} cm^{-1} : 3426.71, 2922.92, 1643.85, 1459.26; ^1H NMR ($CDCl_3$, 400 MHz) δ_H : 13.72 (*s*, 1H, 1-OH), 6.24 (*s*, 1H, H-4), 6.83 (*s*, 1H, H-5), 3.82 (*s*, 3H, 7-O-CH₃), 6.75 (*d*, 1H, $J=10.0$, H-10), 5.59 (*d*, 1H, $J=10.0$, H-11), 1.48 (*s*, 6H, H-13/14), 4.09 (*d*, 2H, $J=6.2$, H-1'), 5.27 (*t*, 1H, $J=5.0$, H-2'), 1.84 (*s*, 3H, H-4'), 1.71 (*s*, 3H, H-5'); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C : 157.9 (C-1), 104.5 (C-2), 159.8 (C-3), 94.2 (C-4), 156.2 (C-4a), 101.8 (C-5), 155.7 (C-5a), 154.7 (C-6), 142.7 (C-7), 61.9 (7-O-CH₃), 137.0 (C-8), 112.1 (C-8a), 181.9 (C-9), 103.7 (C-9a), 115.7 (C-10), 127.2 (C-11), 77.9 (C-12), 28.3 (C-13/14), 26.5 (C-1'), 123.2 (C-2'), 132.2 (C-3'), 18.2 (C-4'), 25.8 (C-5'). The ^1H and ^{13}C NMR spectral data are identical with Wang et al. (2018).

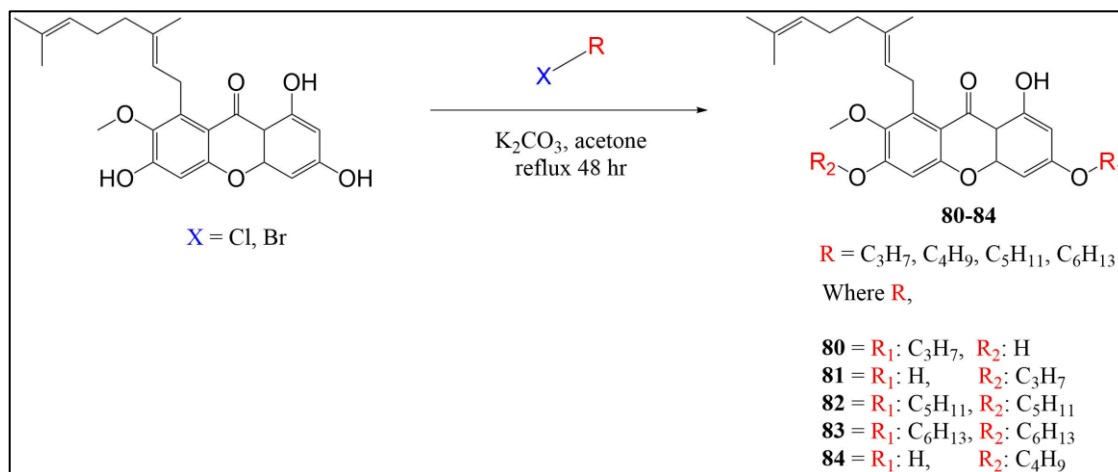
3.5.6 Stigmasterol (59)



White needle crystal; m.p. 175 °C (Lit 174-176 °C) (Chaturvedula & Prakash, 2012); LC-MS (m/z): 412.70; C₂₉H₄₈O; UV-vis (λ_{max}): 203; IR ν_{max} cm⁻¹: 3415.41, 2930.93, 1456.09, 1049.79; ¹H NMR (CDCl₃, 500 MHz) δ_{H} : 5.33 (*t*, 1H, *J*=2.6, H-6), 5.13 (*m*, 1H, H-23), 4.99 (*m*, 1H, H-22), 3.50 (*m*, 1H, OH-3), 1.00 (*s*, 3H, H-18), 0.79-0.83 (*m*, 12H, H-26, H-27, H-21, H-19), 0.69 (*s*, 3H, H-29); ¹³C NMR (CDCl₃, 125 MHz) δ_{C} : 37.3 (C-1), 31.7 (C-2), 71.9 (C-3), 42.3 (C-4), 140.8 (C-5), 121.8 (C-6), 31.9 (C-7), 31.9 (C-8), 50.2 (C-9), 36.6 (C-10), 21.2 (C-11), 39.8 (C-12), 42.4 (C-13), 56.9 (C-14), 24.4 (C-15), 28.9 (C-16), 56.0 (C-17), 12.3 (C-18), 19.1 (C-19), 40.6 (C-20), 21.3 (C-21), 138.4 (C-22), 129.4 (C-23), 51.3 (C-24), 45.9 (C-25), 19.5 (C-26), 21.3 (C-27), 25.5 (C-28), 12.1 (C-29). The ¹H and ¹³C NMR spectral data agree with Kamal et al. (2016).

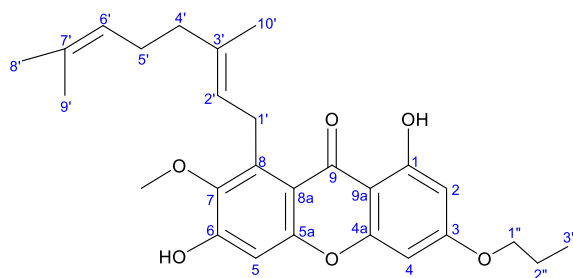
3.6 Structural Modification of Rubraxanthone (2)

The structural modification was followed (Abd Halim et al., 2023) with some modifications. The rubraxanthone derivatives (**80-84**) were synthesized by implementing Williamson ether synthesis with different chain length of primary alkyl halide as shown in Scheme 3.1. Generally, in a 1:3 ratio, rubraxanthone (**2**) was dissolved in 20 mL of acetone, followed by primary alkyl halide. Then, potassium carbonate was added as catalyst. The solution mixture was heated (65 °C) at reflux for 48 hours while stirring. The mixture was allowed to cool to room temperature and then filtered. The filtrate was subsequently cooled until a yellow solid formed. This solid was further purified using column chromatography or preparative thin-layer chromatography. The general procedure for the preparation of **80-84** utilized a different type of alkyl halides (g, mmol) and yields as shown in subsection 3.6.1 to 3.6.5.



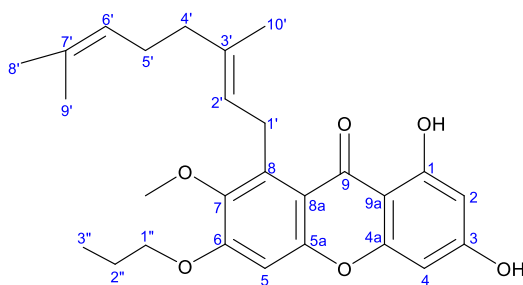
Scheme 3.1 Structural modification of rubraxanthone (**2**)

3.6.1 3-*O*-propyl-rubraxanthone (80)



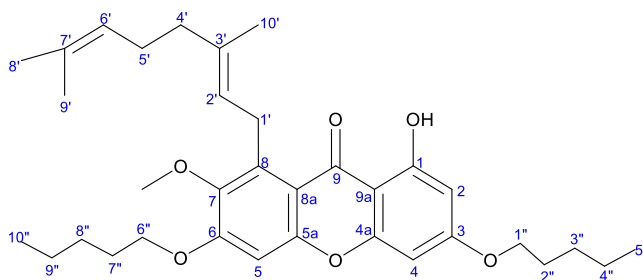
1-Bromopropane (0.092 g, 0.75 mmol); Yellow powder; Yield: 1.1 mg (0.97 %); m.p. 214 °C ; LC-MS (m/z): 452.20; C₂₇H₃₂O₆; UV-vis (λ_{max}): 255, 310; IR ν_{max} cm⁻¹: 3993.13, 2925.16, 1646.22, 1463.71, 1084.54 ; ¹H NMR (acetone-d₆, 400 MHz) δ_{H} : 13.47 (s, 1H, 1-OH), 6.28 (d, 1H, *J*=2.2, H-2), 6.42 (d, 1H, *J*=2.3, H-4), 6.87 (s, 1H, H-5), 3.82 (s, 3H, 7-O-CH₃), 4.13 (d, 2H, *J*=6.5, H-1'), 5.29 (t, 1H, *J*=6.5, 6.6, H-2'), 1.99 (t, 2H, *J*=7.0, 7.3, H-4'), 2.07 (m, 2H, H-5'), 5.05 (t, 1H, *J*=6.9, 7.0, H-6'), 1.57 (s, 3H, H-8'), 1.54 (s, 3H, H-9'), 1.85 (m, 3H, H-10'), 4.10 (t, 2H, *J*=6.5, H-1''), 1.83 (m, 2H, H-2''), 1.06 (t, 3H, *J*=7.4, H-3''); ¹³C NMR (acetone-d₆, 100 MHz) δ_{C} : 163.7 (C-1), 97.1 (C-2), 165.6 (C-3), 91.9 (C-4), 156.9 (C-4a), 101.9 (C-5), 155.5 (C-5a), 156.9 (C-6), 143.8 (C-7), 60.5 (7-O-CH₃), 137.4 (C-8), 111.1 (C-8a), 181.9 (C-9), 103.4 (C-9a), 25.9 (C-1'), 123.8 (C-2'), 134.3 (C-3'), 39.6 (C-4'), 26.4 (C-5'), 124.3 (C-6'), 130.7 (C-7'), 24.8 (C-8'), 16.8 (C-9'), 15.7 (C-10'), 69.9 (C-1''), 22.1 (C-2''), 9.7 (C-3'').

3.6.2 6-*O*-propyl-rubraxanthone (81)



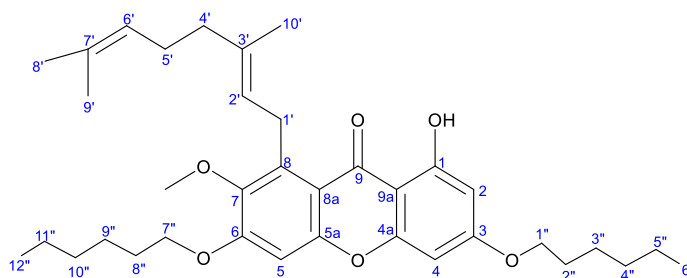
1-Bromopropane (0.092 g, 0.75 mmol); Pale yellow powder; Yield: 15.0 mg (13.26 %); m.p. 215 °C ; LC-MS (*m/z*): 452.20; C₂₇H₃₂O₆; UV-*vis* (λ_{max}): 250, 310; IR ν_{max} cm⁻¹: 3327.40, 2922.17, 1651.48, 1463.06, 1081.02; ¹H NMR (acetone-d₆, 400 MHz) δ_{H} : 13.48 (*s*, 1H, 1-OH), 6.22 (*d*, 1H, *J*=2.1, H-2), 6.33 (*d*, 1H, *J*=2.0, H-4), 6.98 (*s*, 1H, H-5), 3.83 (*s*, 3H, 7-O-CH₃), 4.16 (*d*, 2H, *J*=6.6, H-1'), 5.27 (*t*, 1H, *J*=6.6, 6.7, H-2'), 1.98 (*m*, 2H, H-4'), 2.07 (*m*, 2H, H-5'), 5.06 (*t*, 1H, *J*=6.9, H-6'), 1.57 (*s*, 3H, H-8'), 1.54 (*s*, 3H, H-9'), 1.85 (*s*, 3H, H-10'), 4.20 (*t*, 2H, *J*=6.4, H-1''), 1.93 (*m*, 2H, H-2''), 1.13 (*t*, 3H, *J*=7.4, H-3''); ¹³C NMR (acetone-d₆, 100 MHz) δ_{C} : 163.9 (C-1), 97.9 (C-2), 164.7 (C-3), 92.9 (C-4), 157.1 (C-4a), 99.2 (C-5), 155.4 (C-5a), 158.1 (C-6), 144.4 (C-7), 60.1 (7-O-CH₃), 136.5 (C-8), 111.2 (C-8a), 181.9 (C-9), 102.9 (C-9a), 25.7 (C-1'), 123.8 (C-2'), 134.2 (C-3'), 39.6 (C-4'), 26.4 (C-5'), 124.3 (C-6'), 130.7 (C-7'), 24.9 (C-8'), 16.8 (C-9'), 15.7 (C-10'), 70.4 (C-1''), 22.2 (C-2''), 9.9 (C-3'').

3.6.3 3,6-*O*-pentyl-rubraxanthone (82)



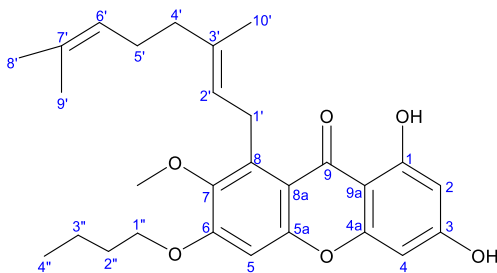
1-Bromopentane (0.116 g, 0.75 mmol); Orange wax; Yield: 19.0 mg (13.81 %); m.p. 199 °C ; LC-MS (*m/z*): 550.35; C₃₄H₄₆O₆; UV-*vis* (λ_{max}): 245, 310; IR ν_{max} cm⁻¹: 2927.57, 1646.03, 1459.04, 1040.81; ¹H NMR (acetone-*d*₆, 400 MHz) δ_{H} : 13.39 (*s*, 1H, 1-OH), 6.19 (*d*, 1H, *J*=2.1, H-2), 6.27 (*d*, 1H, *J*=2.0, H-4), 6.80 (*s*, 1H, H-5), 3.81 (*s*, 3H, 7-O-CH₃), 4.10 (*m*, 2H, H-1'), 5.28 (*t*, 1H, *J*=6.2, 6.6, H-2'), 1.99 (*m*, 2H, H-4'), 2.07 (*m*, 2H, H-5'), 5.05 (*t*, 1H, *J*=6.9, 7.0, H-6'), 1.58 (*m*, 3H, H-8'), 1.54 (*m*, 3H, H-9'), 1.85 (*m*, 3H, H-10'), 4.07 (*m*, 2H, H-1''), 1.55 (*m*, 4H, H-2''/7''), 1.81 (*m*, 2H, H-3''), 1.46 (*m*, 2H, H-4''), 0.97 (*m*, 6H, H-5''/10''), 4.15 (*t*, 2H, *J*=6.4, H-6''), 1.91 (*m*, 2H, H-8''), 1.45 (*m*, 2H, H-9''); ¹³C NMR (acetone-*d*₆, 100 MHz) δ_{C} : 163.6 (C-1), 96.9 (C-2), 165.4 (C-3), 91.8 (C-4), 156.7 (C-4a), 99.0 (C-5), 155.2 (C-5a), 157.9 (C-6), 144.3 (C-7), 60.1 (7-O-CH₃), 136.4 (C-8), 111.1 (C-8a), 181.8 (C-9), 103.4 (C-9a), 25.7 (C-1'), 123.9 (C-2'), 134.1 (C-3'), 39.7 (C-4'), 26.5 (C-5'), 124.3 (C-6'), 130.7 (C-7'), 24.9 (C-8'), 16.8 (C-9'), 15.7 (C-10'), 68.4 (C-1''), 28.1 (C-2''/7''), 28.6 (C-3''), 27.9 (C-4''), 13.4 (C-5''/10''), 68.8 (C-6''), 28.6 (C-8''), 22.2 (C-9'').

3.6.4 3,6-*O*-hexyl-rubraxanthone (83)



1-Bromohexane (0.249 g, 1.50 mmol); Yellow wax; Yield: 115.1 mg (39.8 %); m.p. 214 °C ; LC-MS (*m/z*): 578.35; C₃₆H₅₀O₆; UV-*vis* (λ_{max}): 240, 310; IR ν_{max} cm⁻¹: 2923.01, 1651.99, 1460.11, 1040.15 ; ¹H NMR (acetone-*d*₆, 400 MHz) δ_{H} : 13.43 (*s*, 1H, 1-OH), 6.26 (*d*, 1H, *J*=2.3, H-2), 6.37 (*d*, 1H, *J*=2.2, H-4), 6.93 (*s*, 1H, H-5), 3.83 (*s*, 3H, 7-O-CH₃), 4.14 (*m*, 2H, H-1'), 5.28 (*t*, 1H, *J*=6.13, 6.68, H-2'), 1.99 (*m*, 2H, H-4'), 2.07 (*m*, 2H, H-5'), 5.06 (*t*, 1H, *J*=6.9, H-6'), 1.58 (*s*, 3H, H-8'), 1.54 (*s*, 3H, H-9'), 1.85 (*s*, 3H, H-10'), 4.22 (*t*, 2H, *J*=6.4, H-1''), 1.90 (*m*, 2H, H-2''), 1.39 (*m*, 4H, H-3''/9''), 1.60 (*m*, 2H, H-4''), 1.40 (*m*, 6H, H-5''/11''), 0.94 (*m*, 6H, H-6''/12''), 4.12 (*m*, 2H, H-7''), 1.80 (*m*, 2H, H-8''), 1.51 (*m*, 2H, H-10''); ¹³C NMR (acetone-*d*₆, 100 MHz) δ_{C} : 163.7 (C-1), 97.0 (C-2), 165.6 (C-3), 91.9 (C-4), 156.9 (C-4a), 99.2 (C-5), 155.4 (C-5a), 158.1 (C-6), 144.4 (C-7), 60.1 (7-O-CH₃), 136.5 (C-8), 111.2 (C-8a), 181.9 (C-9), 103.4 (C-9a), 25.7 (C-1'), 123.7 (C-2'), 134.2 (C-3'), 39.6 (C-4'), 26.5 (C-5'), 124.3 (C-6'), 130.7 (C-7'), 24.9 (C-8'), 16.8 (C-9'), 15.7 (C-10'), 68.9 (C-1''), 28.8 (C-2''), 22.4 (C-3''/9''), 25.6 (C-4''), 31.4 (C-5''/11''), 13.4 (C-6''/12''), 68.5 (C-7''), 28.8 (C-8''), 25.5 (C-10'').

3.6.5 6-*O*-butyl-rubraxanthone (84)



1-Chlorobutane (0.070 g, 0.75 mmol); Yellow powder; Yield: 0.6 mg (0.51 %); m.p. 214 °C; LC-MS (*m/z*): 466.25; C₂₈H₃₄O₆; UV-*vis* (λ_{max}): 237, 310; IR ν_{max} cm⁻¹: 3382.95, 2928.54, 1641.85, 1458.69, 1084.41; ¹H NMR (acetone-*d*₆, 500 MHz) δ_{H} : 6.18 (*d*, 1H, *J*=2.3, H-2), 6.29 (*d*, 1H, *J*=2.3, H-4), 6.96 (*s*, 1H, H-5), 3.78 (*s*, 3H, 7-*O*-CH₃), 4.11 (*d*, 2H, *J*=6.3, H-1'), 5.23 (*t*, 1H, *J*=6.2, H-2'), 1.81-1.96 (*m*, 8H, H-4', H-5', H-2'', H-3''), 5.01 (*t*, 1H, *J*=6.9, H-6'), 1.53 (*s*, 3H, H-8'), 1.50 (*s*, 3H, H-9'), 1.85 (*m*, 3H, H-10'), 4.21 (*t*, 2H, *J*=6.3, H-1''), 0.99 (*t*, 3H, *J*=7.2, H-4'').

3.7 Anti-bacterial Assay

3.7.1 Agar Well Diffusion Method

Mueller-Hinton agar was used to culture the bacteria in agar well diffusion method. The bacteria stock turbidity was compared with the cloudiness of the 0.5McFarland standard solution. Bacteria stock (50 μ L) was pipetted onto the agar plate and was spread evenly. Then, 100 μ L of the sample was pipetted into the well and incubated at 37 °C for 24 hours. Ampicillin was used as the positive control while dimethyl sulfoxide (DMSO) was used as the negative control. The crude extract used was at a concentration of 1000 μ g/mL, while the isolated and synthesized compound was 100 μ g/mL. The anti-microbial activity was evaluated by measuring the diameter zone of inhibition (IZD) after incubating the plate for

24 hours and comparing the anti-microbial activity with the positive control. The agar well diffusion method was prepared following Zamakshshari et al. (2022).

3.7.2 Minimum Inhibition Concentration (MIC)

The minimum inhibitory concentration (MIC) values were determined using broth microdilution technique in 96-well microplates in accordance with Nurakmal et al. (2022), with modification. Generally, a two-fold serial dilution method ranging from 1000 µg/mL to 1.95 µg/mL was used to test the sample extract. Meanwhile, the isolated compound was evaluated from 100 µg/mL to 0.195 µg/mL.

Bacteria strains were obtained from 24-hour broth cultures, and suspensions were adjusted according to the turbidity of 0.5McFarland standard. A thousand microlitre (1000 mg/mL) stock solution was prepared by diluting 10 mg of the extracts or 1 mg isolated compound in 1 mL of DMSO. Using a multi-pipettor, a 100 µL of medium (broth) was added into all sterile 96-well microplates. Then, 100 µL of appropriate extract or compound solution was pipetted into the column 1st row and dispended until homogeneous. Following that, 100 µL of the homogeneous solution was transferred from the 1st column row into the 2nd row and dispensed until homogeneous again. The procedure was only repeated to the 10th column, where the remaining two columns were left for control (Appendix F). Five microliter (5 µL) bacteria solution was added to each well except for the control. Finally, the plate was incubated at 37 °C for 24 hours. Turbidity and the presence of a pellet at the well's bottom indicated microbial development. The MIC value was recorded by the lowest reading of the inhibition of bacteria. The MIC samples that showed no signs of bacterial growth were continued for MBC testing.

3.7.3 Minimum Bactericidal Concentration (MBC)

Samples from the MIC investigation that did not show any bacterial growth were withdrawn from each well and transferred onto the surface of the newly produced nutrient agar in disposable petri plates. The petri plates were then inverted and incubated for 24 hours at 37 °C. The MBC was measured by examining the colony growth on each plate after the incubation hours (Nurakmal et al., 2022).

3.8 Statistical Analysis

The anti-microbial testing was done in triplicate. It was represented as the mean $\pm$ standard deviation performed by using Microsoft Excel (Version 2019). Excel is a reliable and convenient tool for calculating mean and standard deviation, especially for basic statistical analysis due to its accuracy and versatility (Ilaboya & Otuario, 2019).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Extraction and Isolation of Secondary Metabolites from *Garcinia dryobalanoides*

Malaysia has a tropical rainforest that consists of diverse tree species. However, many species, including Malaysian *Garcinia* plants, have not yet been fully discovered. With a continuing interest in Malaysian *Garcinia* plants, herein, *Garcinia dryobalanoides*, collected from Semenggoh, Sarawak, was chosen to carry out the detailed studies on the secondary metabolite as well as its antimicrobial activities against several bacteria strains.

The stembark of *G. dryobalanoides* (3.40 kg) had afforded 41.40 g of hexane extract, 161.05 g of ethyl acetate extract and 69.87 g of methanol extract. The isolation and purification of the extracts gave one benzophenone, four xanthones and one triterpene (Figure 4.1). The detailed work on the purification of hexane extract had afforded 2-hydroxy-(4-octyloxy)benzophenone (**76**) (29.4 mg), 9-hydroxycalabaxanthone (**79**) (32.0 mg) and stigmasterol (**59**) (11.9 mg). Meanwhile, for the ethyl acetate extract yielded rubraxanthone (**2**) (4202.0 mg), 8-deoxygartanin (**77**) (12.2 mg) and isocowanol (**78**) (5.6 mg). Rubraxanthone (**2**), the predominant compound in the plant, was also isolated from the methanol extract. Astonishingly, compound **76** was isolated for the first time in the *Garcinia* genus. Previously, Khamidullina et al. (2005) were the first to isolate compound **76** from *Pinus sibirica* shell.

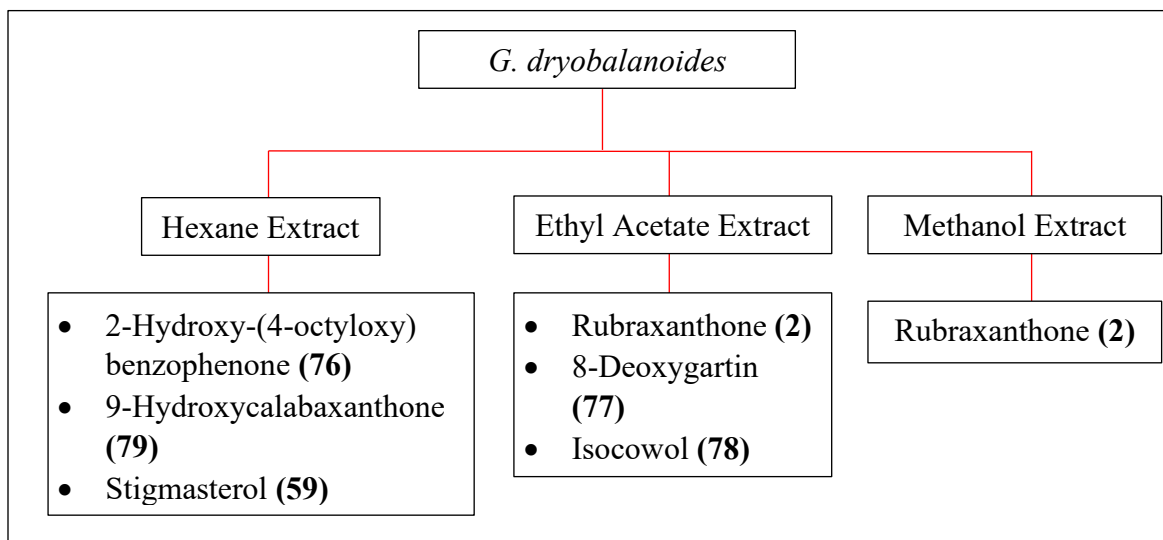


Figure 4.1: Secondary metabolites isolated from *G. dryobalanoides*

4.2 Characterization of Secondary Metabolites from *Garcinia dryobalanoides*

4.2.1 Characterization of 2-Hydroxy-(4-octyloxy)benzophenone (76)

2-Hydroxy-(4-octyloxy)benzophenone (76) was obtained from hexane extract as a light yellowish gum. The molecular formula was determined to be $C_{21}H_{26}O_3$, as supported by the LC-MS spectrum (Figure 4.2) which showed a molecular peak at m/z 326.45.

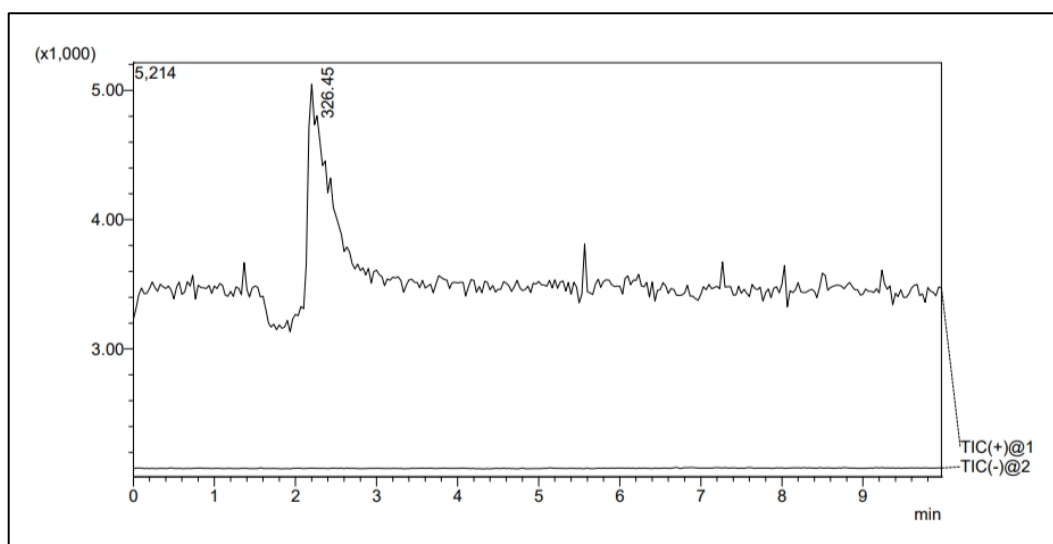


Figure 4.2: LC-MS of compound 76

Aside from that, the functional group of **76** was determined from the FT-IR spectrum (Figure 4.3). They are 2923.72 cm^{-1} , 1618.09 cm^{-1} , 1255.52 cm^{-1} , which indicate the presence of C-H stretch, C=O stretch and C-O stretch, respectively. While an OH group was expected to be evident in the spectrum, however, no corresponding peak was observed. This might be due to the rapid proton exchange of hydroxyl proton (Novakovic et al., 2021). The UV spectrum, on the other hand, revealed maxima at λ_{max} 285 nm consistent with the typical chromophore molecules with conjugated double bonds on aromatic rings due to the presence of π - π^* electron excitation (Acuna et al., 2009).

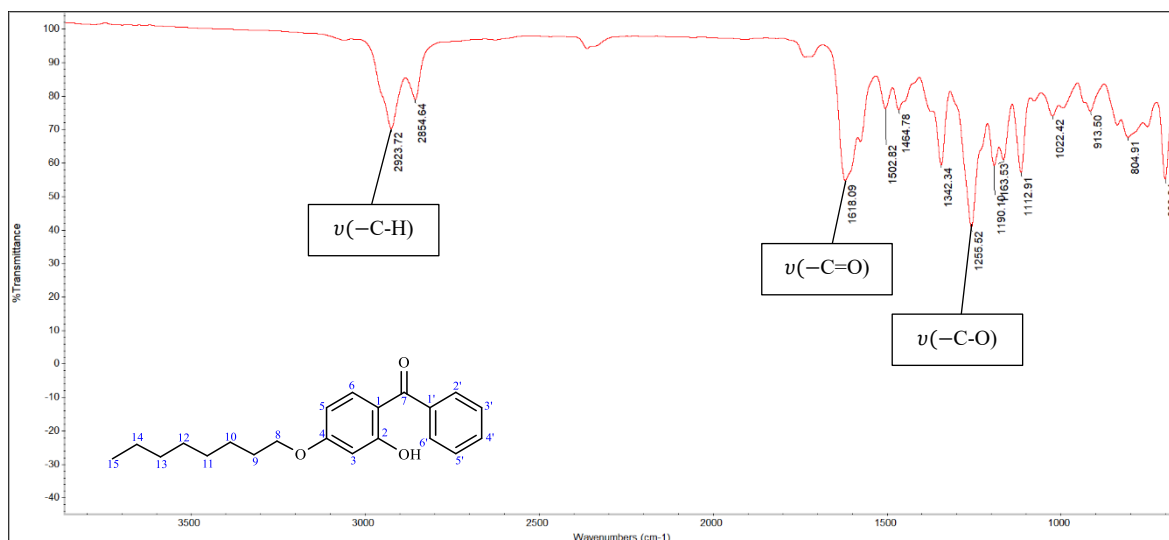


Figure 4.3: FT-IR of compound **76**

The ^1H NMR spectrum (Figure 4.5) signals at δ 7.48 (*m*, 1H, H-6), δ 7.62 (*m*, 2H, H-2', H-6'), δ 7.53 (*m*, 2H, H-3', H-5'), δ 7.61 (*m*, 1H, H-4'), δ 6.50 (*d*, 1H, $J=2.4$, H-3) and δ 6.45 (*dd*, 1H, $J=2.4$, 9.0, H-5) represents the aromatic protons. The HSQC (Figure 4.7) showed that the overlapped methine carbons at δ 128.5 (C-2' and C-6') in the phenyl group were connected to the overlapped protons at δ 7.62 (H-2' and H-6'). Meanwhile, the methine carbons at δ 128.0 (C-3' and C-5') correlated with the protons at δ 7.53 (H-3' and H-5'). In the HMBC spectrum (Figure 4.10), δ 7.48 (H-6) and δ 7.62 (H-2' and H-6') have ^3J correlations with δ 200.1 (C-7) deduced that both phenyl attachment was connected to C-7. This shows that the compound has a benzophenone skeletal structure (Pailee et al., 2018).

Aside from that, there is a pair of triplets at δ 4.04 (H-8) and δ 0.93 (H-15) along with multiplet in the range δ 1.31 to δ 1.79 (H-9 to H-14) shown in the ^1H NMR spectra, suggesting the aliphatic chain in the structure (Simpson, 2017). The methine moiety of the aliphatic chain was deduced to be adjacent to each other based on the ^1H - ^1H COSY spectrum (Figure 4.9). The HMBC cross-peaks of H-8 with C-4 (δ 166.0) in ^3J correlations confirmed unambiguously the positions of the aliphatic chain at C-4. Conversely, δ 6.50 (H-3) has ^2J correlations with δ 165.8 (C-2) and δ 7.48 (H-6) has ^3J correlations with δ 165.8 (C-2), suggesting the attachment of OH at C-2.

The combined application of ^{13}C NMR and DEPT spectra of compound **76** in Figure 4.6 and Figure 4.8 elucidates the presence of 19 carbons that consist of one methyl (δ 13.1), seven methylene (δ 22.3, 31.6, 29.0, 28.9, 25.7, 28.7, 68.2), six methine (δ 101.3, 107.2, 135.1, 128.5, 128.0, 131.3) and five quaternary carbons (δ 112.8, 165.8, 166.0, 200.1, 138.3). A prominent downfield signal at δ 200.1 (C-7) was attributed to a carbonyl carbon, indicative of a benzophenone scaffold. The carbonyl carbon was located at the downfield region

because the carbon atom was deshielded due to the strong electron-withdrawing nature of the oxygen atom in the carbonyl group (Sahariah & Sarma, 2020).

A comprehensive analysis of 1D and 2D NMR data (Table 4.1), along with FT-IR, LC-MS, and UV-*vis* data established the 2D structure of **76** (Figure 4.4) was identified as 2-hydroxy-(4-octyloxy)benzophenone (**76**) (Khamidullina et al., 2005).

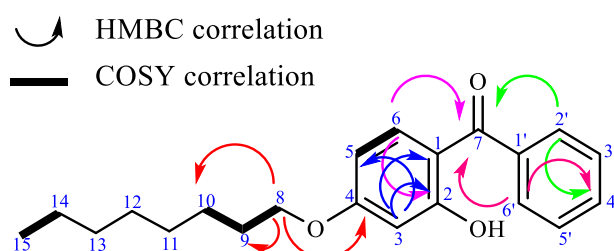


Figure 4.4: HMBC and COSY correlation in compound **76**

Table 4.1: 1D and 2D-NMR assignments of 2-hydroxy-(4-octyloxy)benzophenone (**76**)

Position	¹ H NMR	¹³ C NMR	¹³ C NMR*	DEPT	HMBC
1		112.8	113.0	C	
2		165.8	166.4	C	
3	6.50, <i>d</i> , <i>J</i> =2.4 Hz, 1H	101.3	101.5	CH	C-5, C-1, C-2
4		166.0	165.9	C	
5	6.45, <i>dd</i> , <i>J</i> =2.4, 9.0 Hz, 1H	107.2	107.9	CH	C-3, C-1, C-4
6	7.48, <i>m</i> , 1H	135.1	135.3	CH	C-2, C-7
7		200.1	200.1	C	
8	4.04, <i>t</i> , <i>J</i> = 6.4, 6.5 Hz, 2H	68.2	68.6	CH ₂	C-10, C-9, C-4
9	1.79, <i>m</i> , 2H	28.7	29.0	CH ₂	C-10, C-11, C-8
10	1.48, <i>m</i> , 2H	25.7	26.0	CH ₂	C-9, C-11, C-12
11	1.36, <i>m</i> , 4H	28.9	29.8	CH ₂	C-9, C-13
12		29.0	29.4	CH ₂	C-11, C-13
13	1.31, <i>m</i> , 2H	31.6	31.9	CH ₂	C-11, C-12
14	1.32, <i>m</i> , 2H	22.3	22.8	CH ₂	C-11, C-12, C-13
15	0.93, <i>t</i> , <i>J</i> = 6.9, 7.0 Hz, 3H	13.1	14.2	CH ₃	C-14, C-13
1'		138.3	131.5	C	
2'/6'	7.62, <i>m</i> , 2H	128.5	128.9	CH	C-4', C-7
3'/5'	7.53, <i>m</i> , 2H	128.0	128.4	CH	C-1'
4'	7.61, <i>m</i> , 1H	131.3	138.4	CH	C-2'

¹³C NMR* references adapted from Khamidullina et al. (2005)

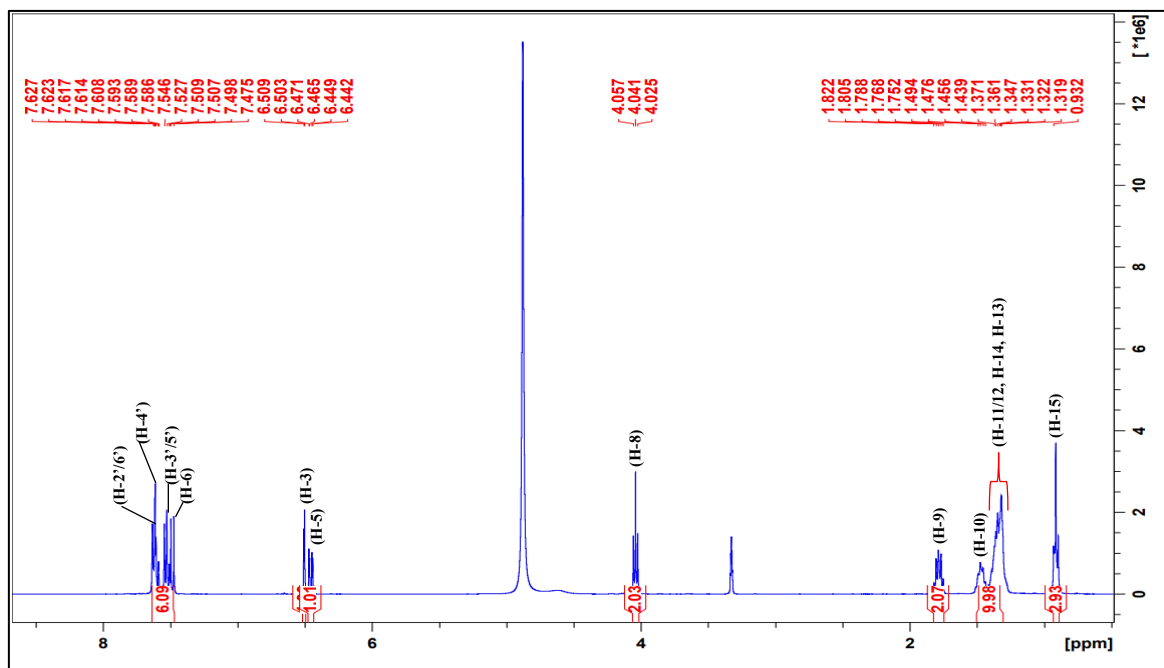


Figure 4.5: ¹H NMR of compound 76

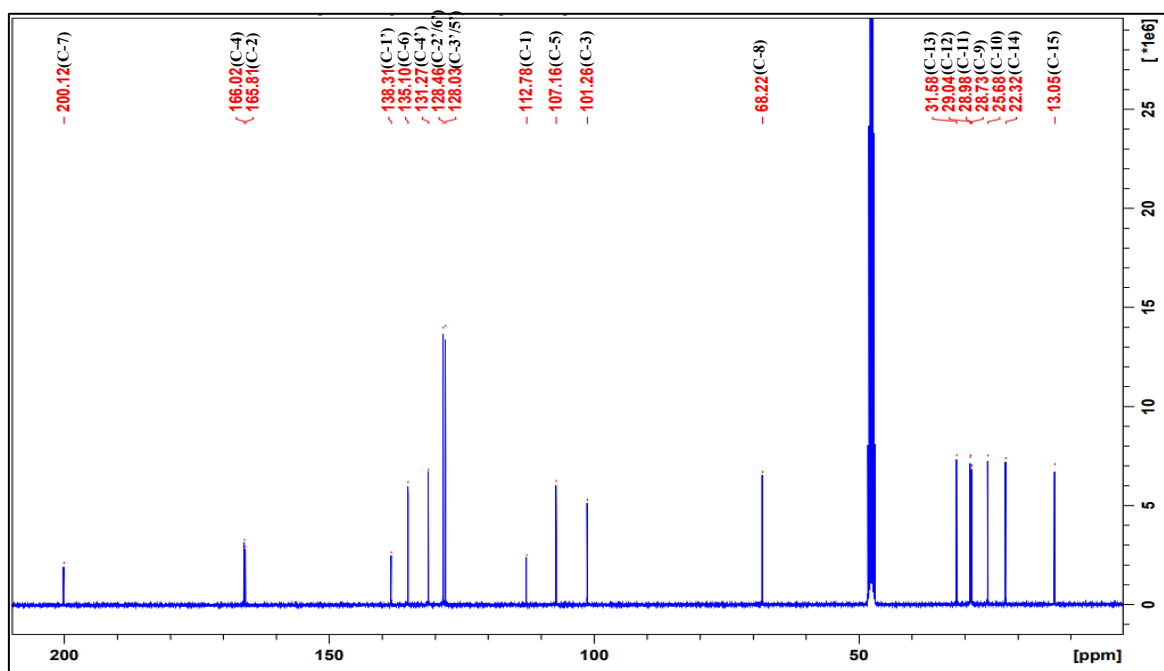


Figure 4.6: ¹³C NMR of compound 76

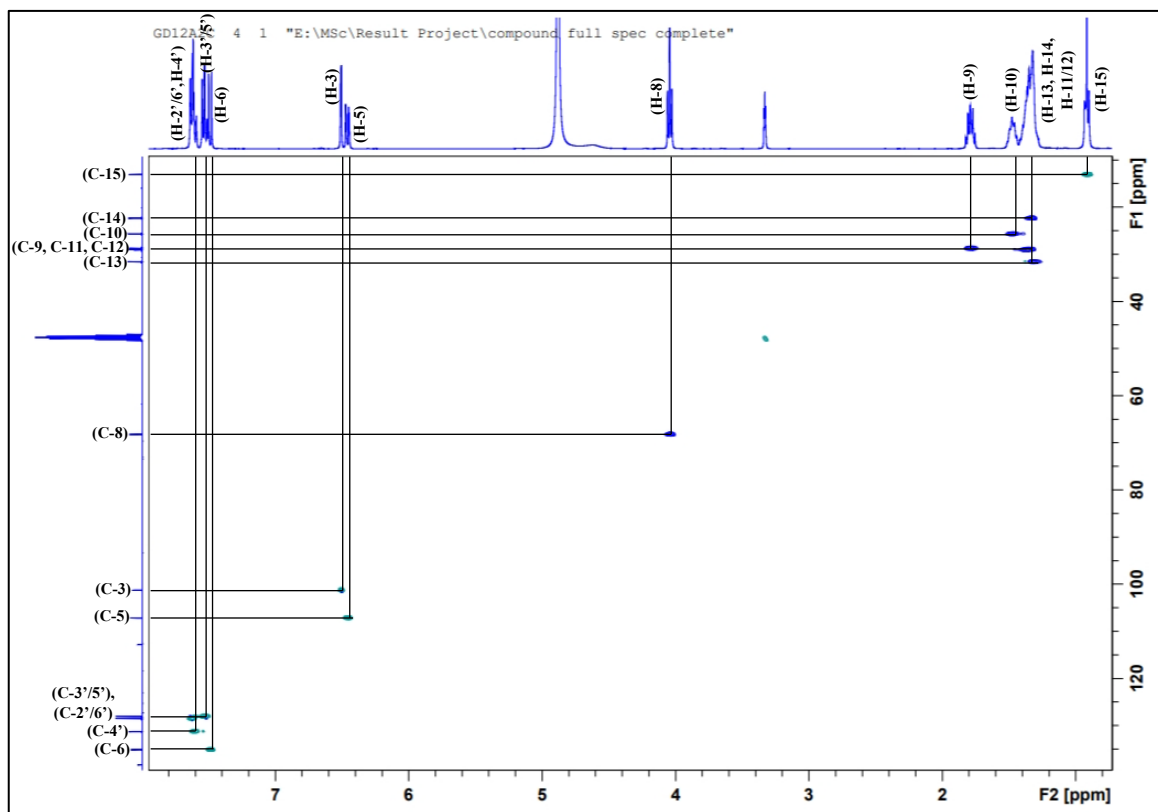


Figure 4.7: HSQC spectra of compound 76

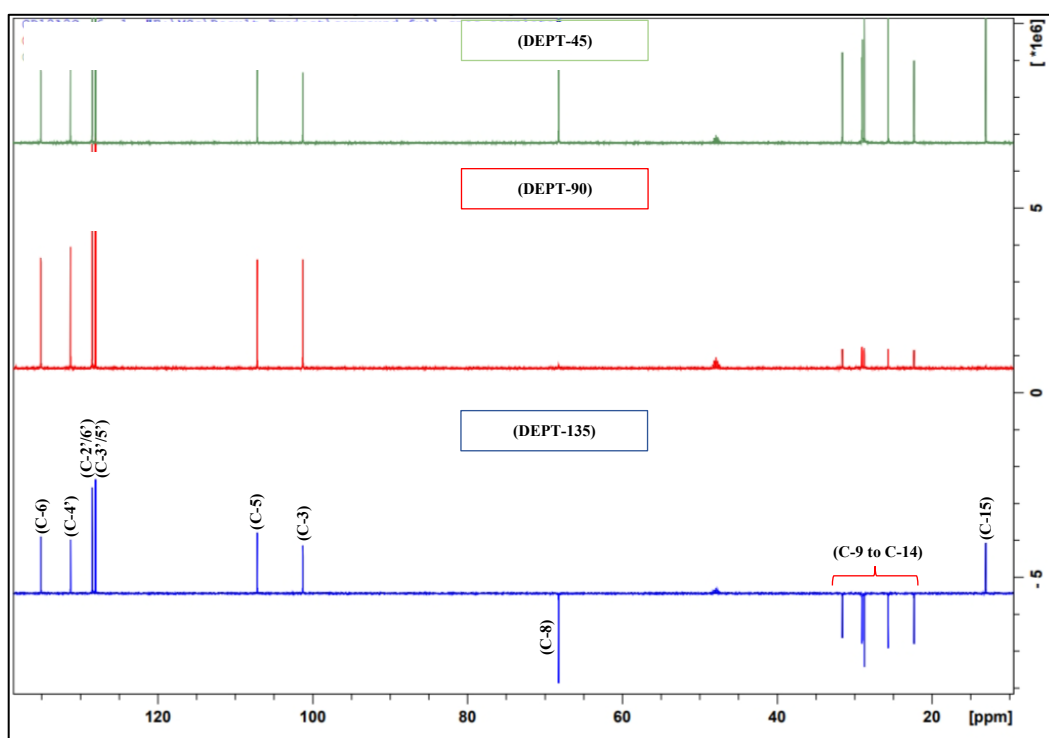


Figure 4.8: DEPT spectra of compound 76

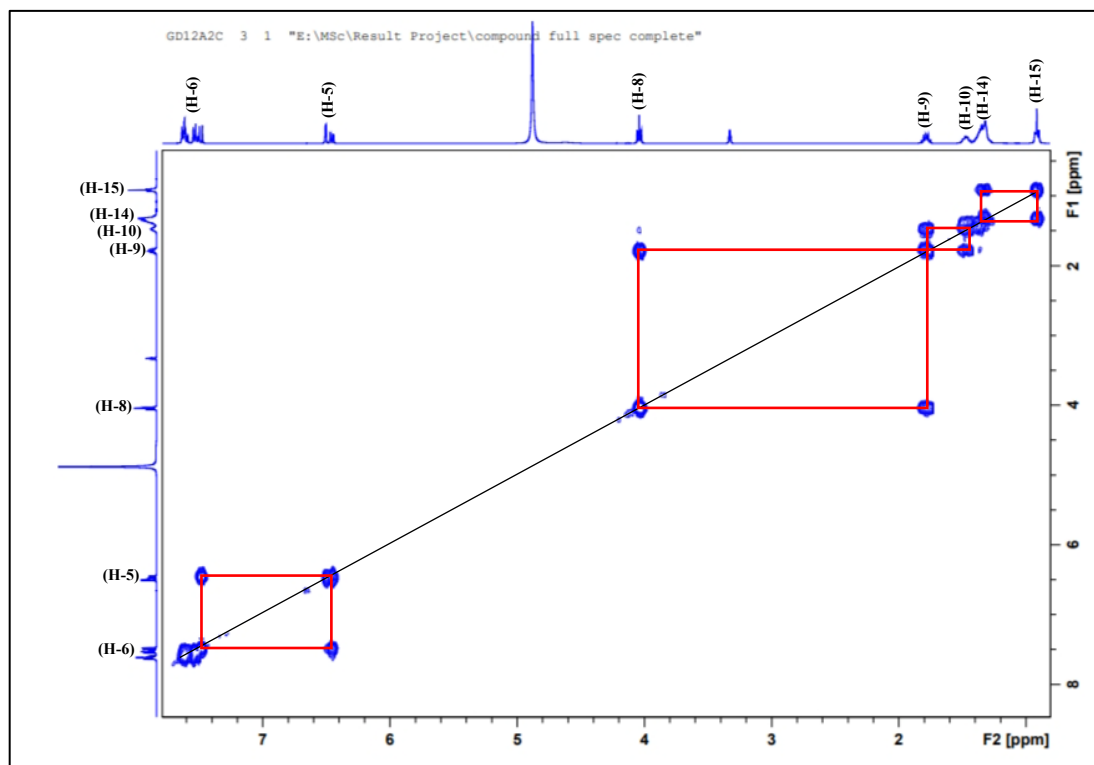


Figure 4.9: COSY spectra of compound **76**

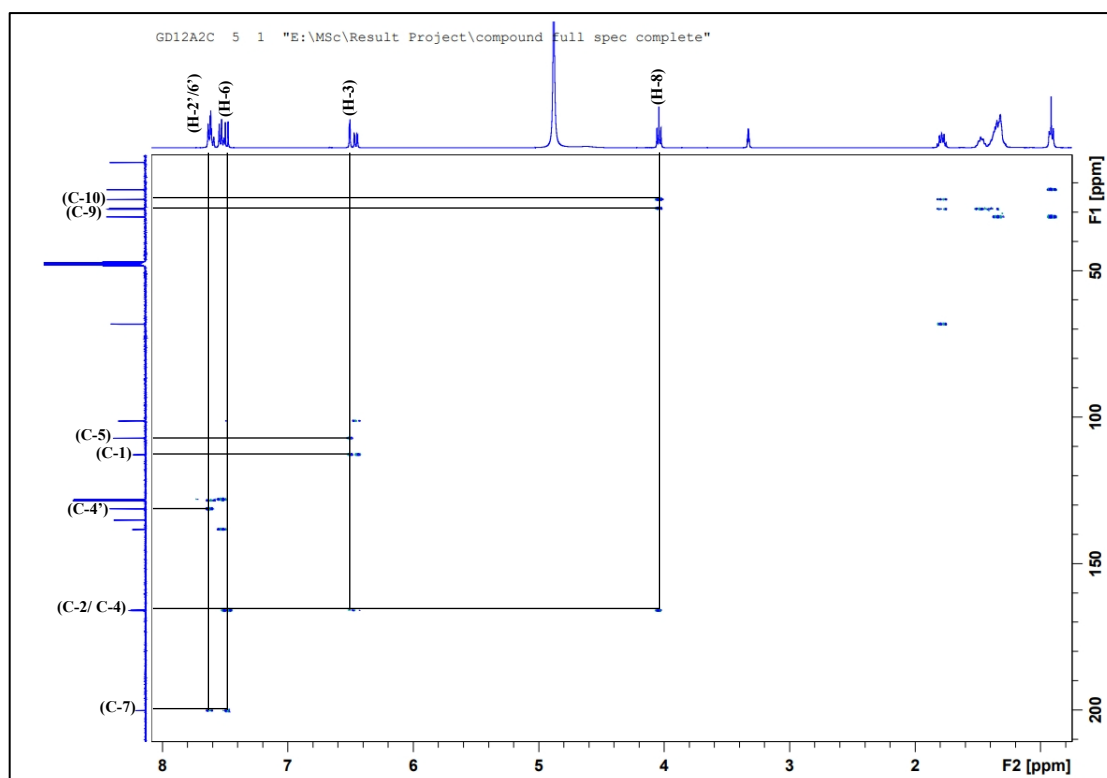


Figure 4.10: HMBC spectra of compound **76**

4.2.2 Characterization of Rubraxanthone (2)

Rubraxanthone (**2**) was isolated as a yellow crystal with a melting point of 205 °C (Lit 207-208 °C) (Thuy et al., 2022) from ethyl acetate extract. The molecular formula $C_{24}H_{24}O_6$ was deduced from LC-MS (molecular peak at m/z 410.45) (Figure 4.11).

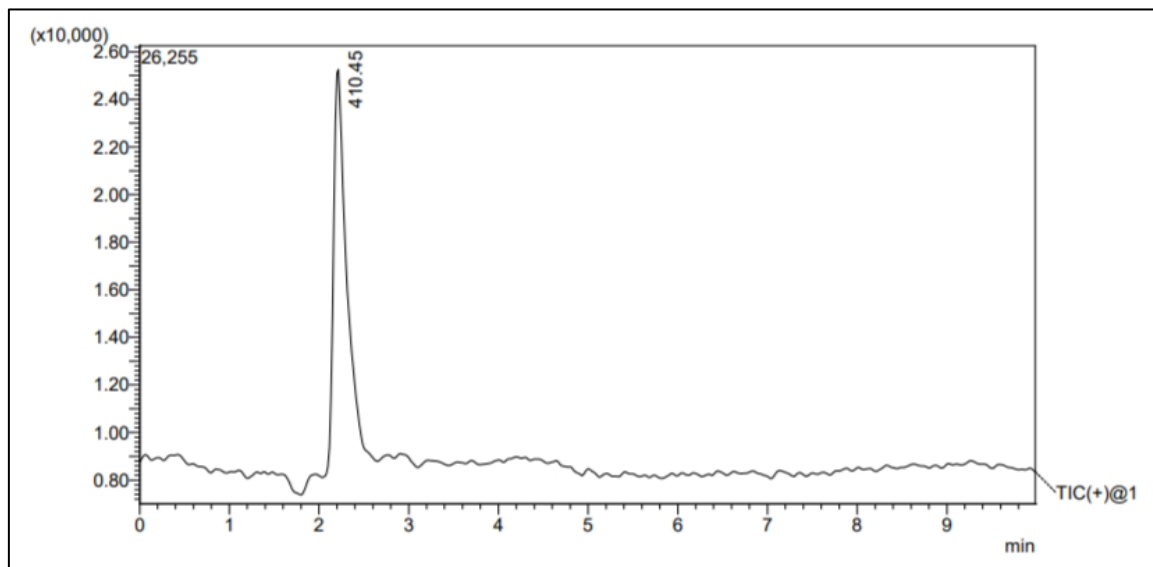


Figure 4.11: LC-MS of compound **2**

The FT-IR spectrum exhibited bands for OH stretch (3421.31 cm^{-1}), C-H stretch (2911.68 cm^{-1}), C=O stretch (1642.94 cm^{-1}), and C=C stretch (1463.36 cm^{-1}) and C-O stretch (1074.44 cm^{-1}) (Figure 4.12). The UV spectrum showed absorption bands at 240 and 310 nm, which indicate the conjugation of aromatic ring and conjugated carbonyl in the structure (Zhang et al., 2024).

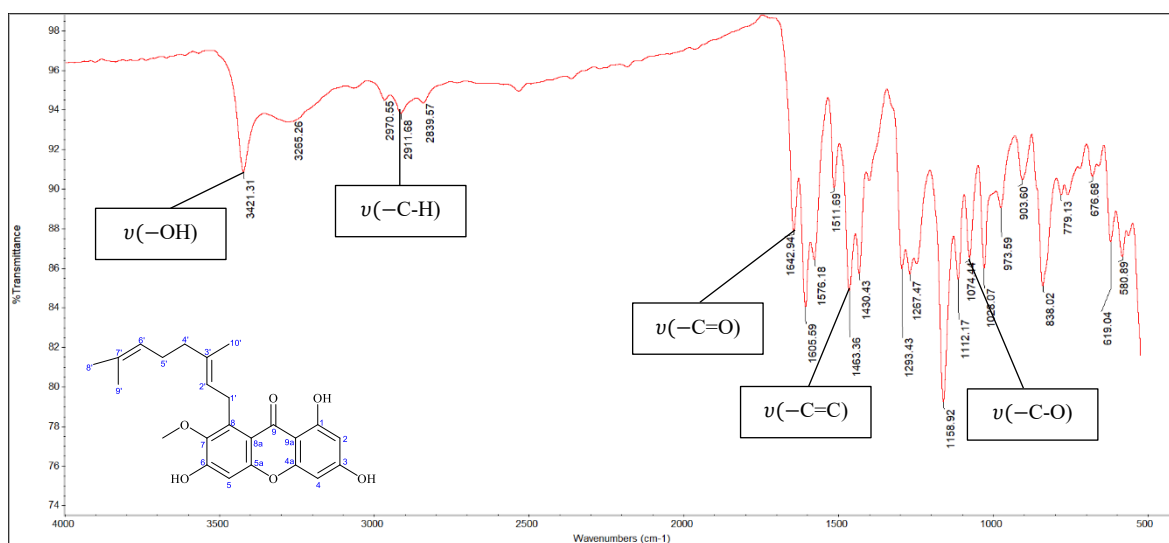


Figure 4.12: FT-IR of compound 2

The ^1H NMR of compound **2** revealed the presence of chelated hydroxyl group at δ 13.47 (1H, s). The chelated hydroxyl proton signal (OH-1) exhibited a downfield shift in the NMR spectrum, attributable to the electron-withdrawing inductive effect of the adjacent carbonyl group (Yuanita et al., 2023). Aside from that, three aromatic protons at δ 6.73 (1H, s); 6.23 (1H, *d*, $J = 2.2$ Hz) and 6.15 (1H, *d*, $J = 2.2$ Hz) were attributed to H-5, H-4, and H-2, respectively, based on the HMBC spectrum (Table 4.2). The coupling constant between H-2 and H-4 was determined at 2.2 Hz, which indicates their *meta* position (Sukandar et al., 2019). Aside from that, a methoxy group at δ 3.79 (3H, s, 7-O-CH₃) also were shown in the ^1H NMR spectrum. Following that, compound **2** exhibited characteristic signals for a geranyl moiety. The presence of geranyl side chain was identified from the resonance of two olefinic methine group [δ 5.27 (1H, *t*, H-2'), δ 5.00 (*t*, H-6')], three methyl [δ 1.53 (3H, s, H-8'), δ 1.49 (3H, s, H-9'), δ 1.80 (3H, s, H-10')] and three methylene [δ 4.07 (2H, *d*, H-1'), δ 1.97-2.02 (4H, *m*, H-4', H-5')] (Thuy et al., 2022).

In the HMBC spectrum, the chelated hydroxyl group observed at δ 13.47 showed correlations with aromatic carbons at δ 163.9 (C-1), δ 97.8 (C-2), δ 102.9 (C-9a) in ^2J to ^3J correlation confirming their location at C-1 of the xanthone nucleus. The ^3J correlation between the methoxy group at δ 3.79 and the carbon signal at δ 143.6 (C-7) in the HMBC spectrum suggests that the methoxy group's position is attached at C-7. Meanwhile, the position of geranyl substituent at C-8 is suggested by a correlation of δ 4.07 (H-1') *via* ^2J and ^3J correlation with C-8 (δ 137.2), C-7 (δ 143.6), and C-8a (δ 111.1).

The ^{13}C NMR and DEPT spectra of compound **2** revealed the presence of 24 carbons that consist of four methyl, three methylene, five methine, and 11 quaternary carbons.

After combining the spectral data, this compound was identified as rubraxanthone (**2**) previously isolated by Thuy et al. (2022). The ^1H and ^{13}C NMR spectra were shown in Figure 4.14 and Figure 4.15, meanwhile, the HMBC correlation was shown in Figure 4.13. The 2D-NMR spectra of the compound was shown in Appendix A.

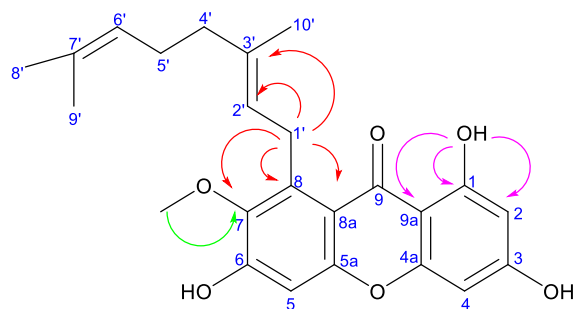


Figure 4.13: HMBC correlation in compound **2**

Table 4.2: 1D and 2D-NMR assignments of rubraxanthone (**2**)

Position	¹ H NMR	¹³ C NMR	¹³ C NMR*	DEPT	HMBC
1-OH	13.47, <i>s</i> , 1H	163.9	162.7	C	C-2, C-9a, C-1
2	6.15, <i>d</i> , <i>J</i> =2.2 Hz, 1H	97.8	98.3	CH	C-4, C-9a, C-1, C-3
3		164.3	163.9	C	
4	6.23, <i>d</i> , <i>J</i> =2.2 Hz, 1H	92.9	93.4	CH	C-2, C-9a, C-4a, C-3
4a		156.9	157.0	C	
5	6.73, <i>s</i> , 1H	101.9	103.9	CH	C-8a, C-7, C-5a, C-6, C-9
5a		155.3	155.8	C	
6		156.4	154.7	C	
7		143.6	142.8	C	
7-O-CH ₃	3.79, <i>s</i> , 3H	60.5	62.0	CH ₃	C-7
8		137.2	137.2	C	
8a		111.1	112.3	C	
9		181.7	181.9	C	
9a		102.9	101.7	C	
1'	4.07, <i>d</i> , <i>J</i> =6.4 Hz, 2H	25.9	26.5	CH ₂	C-8a, C-2', C-3', C-8, C-7
2'	5.27, <i>t</i> , <i>J</i> =5.6, 6.4 Hz, 1H	124.0	123.2	CH	C-10', C-4', C-1', C-8
3'		134.1	135.7	C	
4'	1.97, <i>m</i> , 2H	39.6	39.7	CH ₂	C-10', C-5', C-2', C-6', C-3'
5'	2.02, <i>m</i> , 2H	26.4	26.5	CH ₂	C-4', C-6', C-7', C-3'
6'	5.00, <i>t</i> , <i>J</i> =6.6, 6.8 Hz, 1H	124.3	124.3	CH	C-9', C-8'
7'		130.6	131.3	C	
8'	1.53, <i>s</i> , 3H	24.9	25.6	CH ₃	C-9', C-6', C-7'
9'	1.49, <i>s</i> , 3H	16.9	17.6	CH ₃	C-8', C-6', C-7'
10'	1.80, <i>s</i> , 3H	15.8	16.5	CH ₃	C-4', C-2', C-3'

¹³C NMR*references adapted from Thuy et al. (2022)

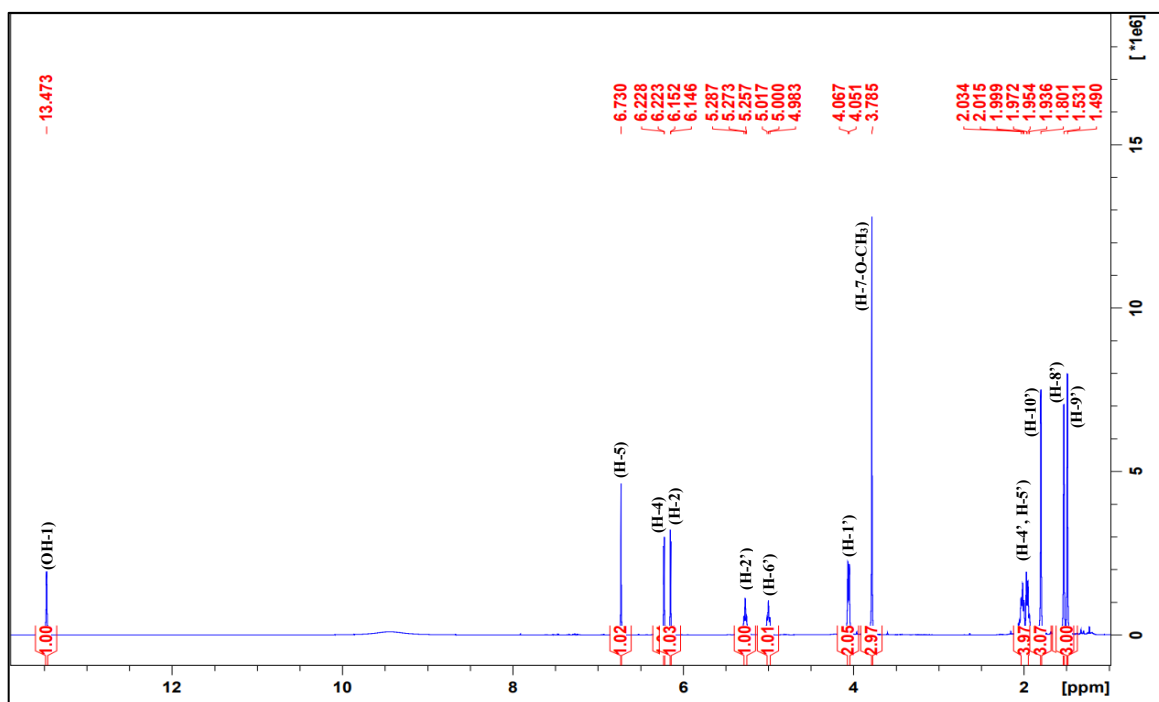


Figure 4.14: ¹H NMR of compound 2

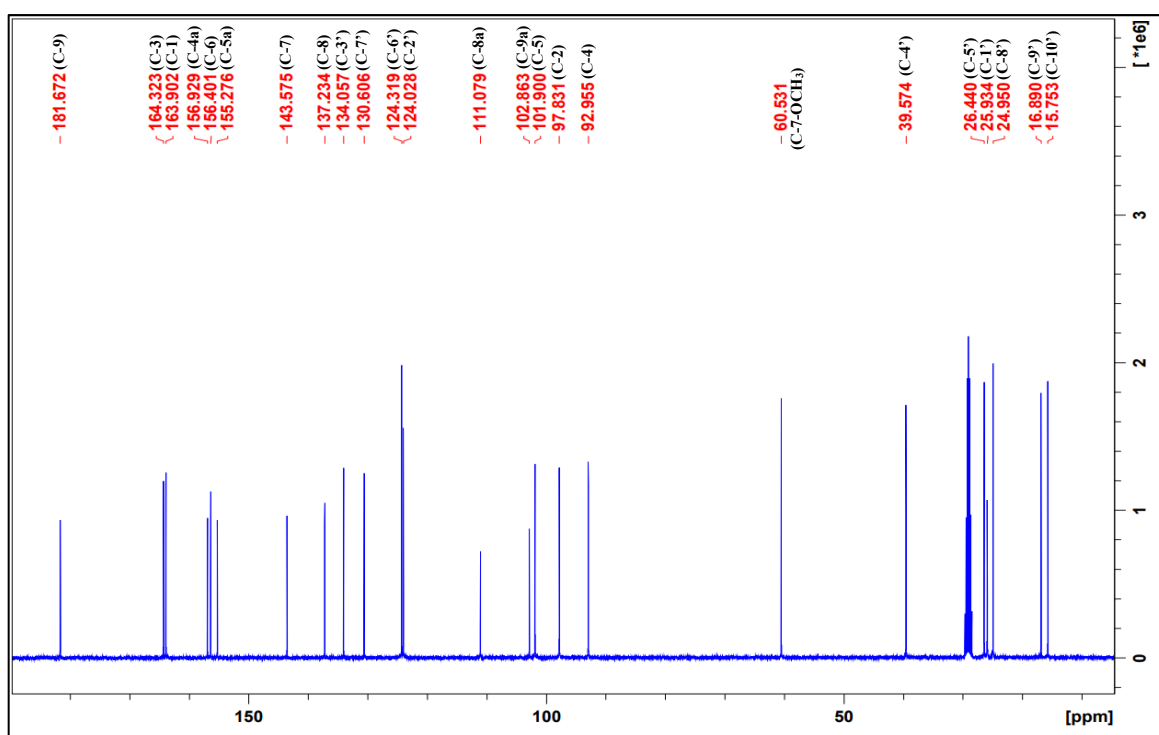


Figure 4.15: ¹³C NMR of compound 2

4.2.3 Characterization of 8-Deoxygartanin (77)

8-Deoxygartanin (**77**) was obtained from the ethyl acetate extract. It is a yellow amorphous powder with a melting point of 167 °C (Lit 165-167 °C, Karunakaran et al., 2022). A molecular formula of $C_{23}H_{24}O_5$ was deduced by the combination of ^1H and ^{13}C NMR and LC-MS data [m/z 380.45 (calculated 380.44)] (Figure 4.16).

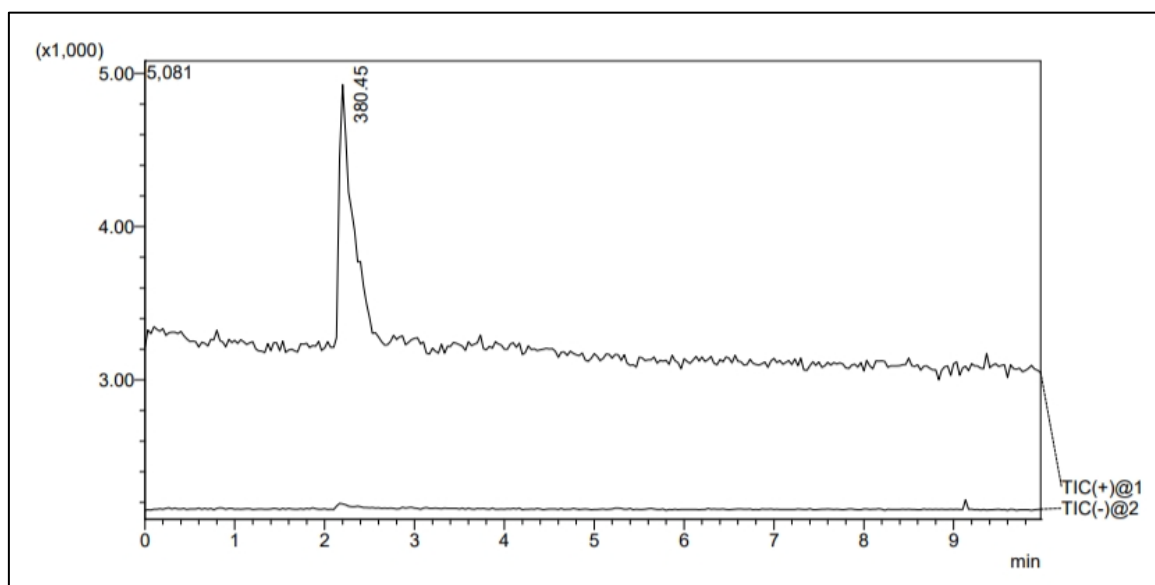


Figure 4.16: LC-MS of compound **77**

The FT-IR spectrum in Figure 4.17 displayed stretching bands of the OH group (3269.39 cm^{-1}), C-H stretch (2916.54 cm^{-1}), C=O group (1638.22 cm^{-1}), aromatic C=C (1476.36 cm^{-1}) and C-O stretch (1093.33 cm^{-1}). The UV λ_{max} absorptions bands at 265, 315 and 385 nm represent typical of a xanthone skeletal structure (Ngoupayo et al., 2009).

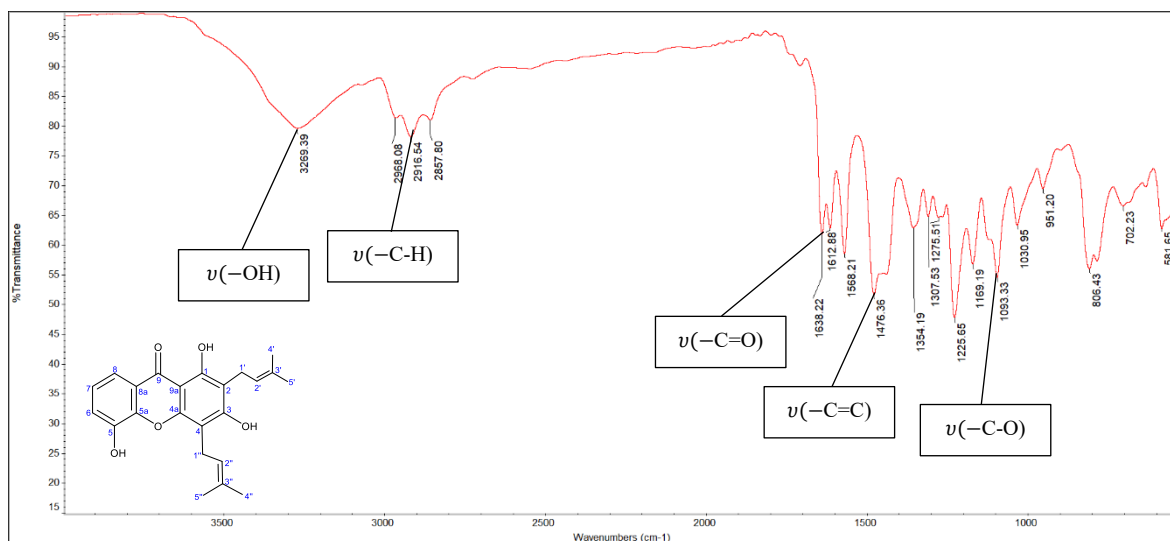


Figure 4.17: FT-IR of compound 77

The ^1H NMR spectrum of **77** exhibited a downfield singlet at δ 13.29 for the chelated hydroxyl group attached to the xanthone ring. The positioning of the chelated hydroxyl group at δ 159.7 (C-1) due to ^2J to ^3J correlation of 1-OH with three quaternary aromatic carbon δ 159.7 (C-1), δ 111.3 (C-2), δ 104.0 (C-9a) shown in the HMBC spectrum (Wang et al., 2018) (Figure 4.18).

A group of signals comprised of a triplet at δ 5.25 (2H, $J=7.0, 7.1$, H-2'/2''), two pair of doublets at δ 3.45 (2H, $J=6.9$, H-1') and δ 3.59 (2H, $J=7.1$, H-1'') along with three singlets at δ 1.80 (H-4'), δ 1.67 (H-5'/5'') and δ 1.89 (H-4'') shows the presence of two prenyl group in the structure. The attachment of the prenyl group in the structure was supported by a long-range coupling between the protons and carbons *via* HMBC analysis. The ^2J to ^3J correlation between δ 3.45 (H-1') with carbon δ 159.7 (C-1), δ 111.3 (C-2), δ 161.8 (C-3) proves the attachment of one prenyl group at C-2. Meanwhile, the ^2J to ^3J correlation of δ 3.59 (H-1'') with carbon δ 107.2 (C-4), δ 154.4 (C-4a), and δ 161.8 (C-3) proves the other attachment of prenyl group at C-4.

A doublet of doublet splitting pattern at δ 7.37 (1H, $J=3.02, 9.0$ Hz, H-6) was found to be ortho-coupled with δ 7.48 (1H, $J=9.0$ Hz, H-7) and meta-coupled with δ 7.59 (1H, $J=3.01$ Hz, H-8). The coupling between olefinic proton was also deduced from the COSY spectrum. In the COSY spectrum, H-7 and H-8 are found to correlate with each other. The attachment of the hydroxy group at C-5 due to quaternary carbon δ 151.2 (C-5) appears at the downfield carbon spectrum (Raksat et al., 2019).

Examination of 1D (Figure 4.19 and Figure 4.20) and 2D NMR (Table 4.3 and Appendix B) with other spectral analyses of **77** associated with reported data Nguyen et al. (2003) confirmed that compound **77** was 8-deoxygartanin.

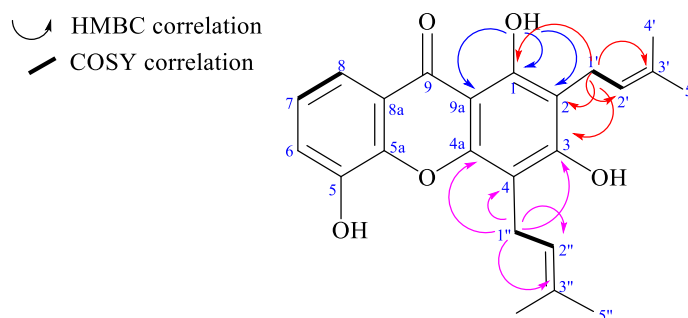


Figure 4.18: HMBC and COSY correlation in compound **77**

Table 4.3: 1D and 2D-NMR assignments of 8-deoxygartanin (**77**)

Position	¹ H NMR	¹³ C NMR	¹³ C NMR*	DEPT	HMBC
1-OH	13.29, <i>s</i> , 1H	159.7	159.8	C	C-9a, C-2, C-1
2		111.3	111.6	C	
3		161.8	162.0	C	
4		107.2	107.8	C	
4a		154.4	153.9	C	
5		151.2	147.6	C	
5a		155.1	146.8	C	
6	7.37, <i>dd</i> , <i>J</i> = 3.0, 9.0 Hz, 1H	125.4	124.9	CH	C-8, C-5, C-5a
7	7.48, <i>d</i> , <i>J</i> = 9.0 Hz, 1H	120.2	121.6	CH	C-8a, C-5, C-5a, C-9
8	7.59, <i>d</i> , <i>J</i> =3.0 Hz, 1H	109.7	116.6	CH	C-7, C-5, C-5a, C-9
8a		122.0	122.5	C	
9		182.0	182.5	C	
9a		104.0	104.2	C	
1'	3.45, <i>d</i> , <i>J</i> =6.9 Hz, 2H	22.6	22.8	CH ₂	C-2, C-2', C-3', C-1, C-3
2' & 2''	5.25, <i>t</i> , <i>J</i> =7.0, 7.1 Hz, 2H	123.5	123.4-123.6	CH	C-4'', C-5', C-5''
3'		132.9	133.0	C	
4'	1.80, <i>s</i> , 3H	18.4	26.34	CH ₃	C-5', C-2', C-3'
5' & 5''	1.67, <i>s</i> , 6H	26.3	18.4-18.5	CH ₃	C-4', C-4'', C-2', C-2'', C-3'', C-3'
1''	3.59, <i>d</i> , <i>J</i> =7.1 Hz, 2H	22.9	22.5	CH ₂	C-4, C-2'', C-3'', C-4a, C-3
3''		132.7	132.9	C	
4''	1.89, <i>s</i> , 3H	18.5	26.26	CH ₃	C-5'', C-2'', C-3''

¹³C NMR* references adapted from Nguyen et al. (2003)

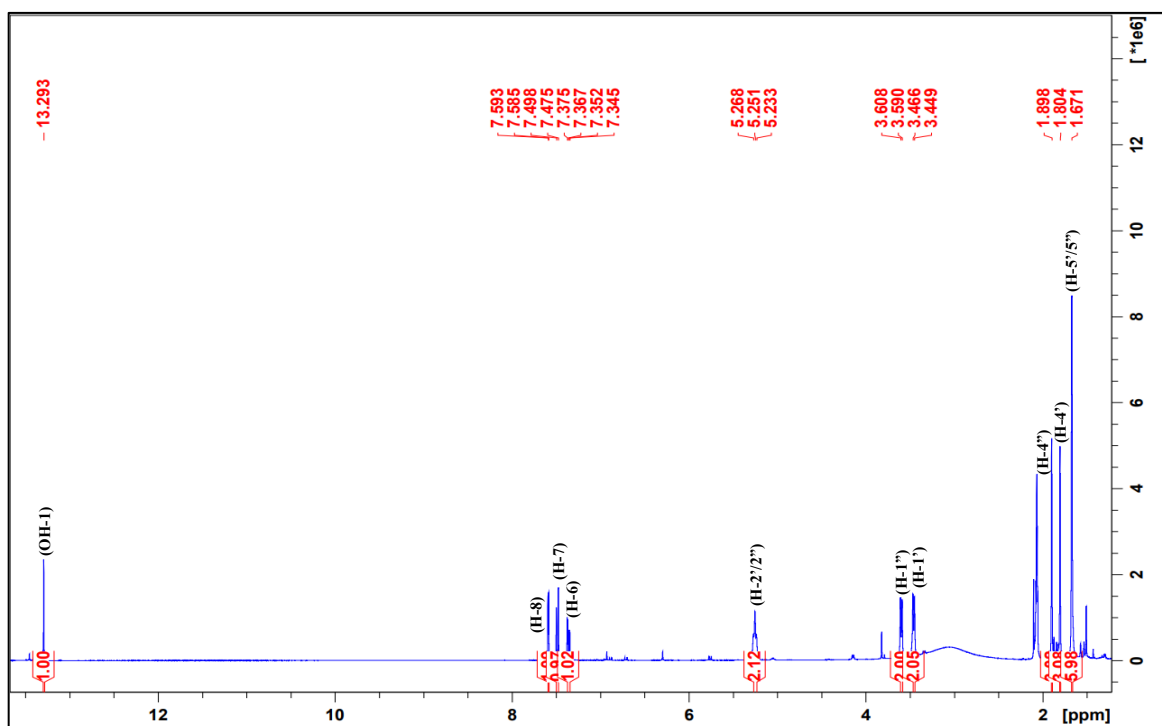


Figure 4.19: ¹H NMR of compound 77

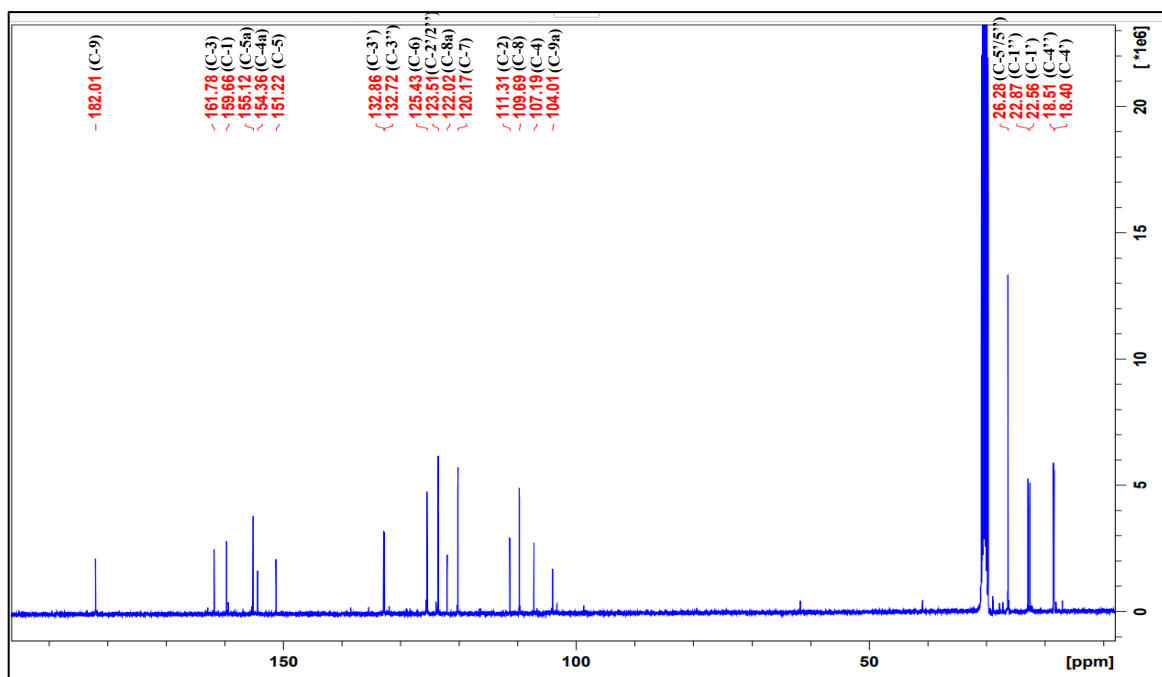


Figure 4.20: ¹³C NMR of compound 77

4.2.4 Characterization of Isocowanol (78)

Isocowanol (**78**), an orange gummy compound, has a molecular formula of $C_{29}H_{34}O_7$. The molecular weight at m/z 494.60 (calculated 494.58) of compound **78** was determined by LCMS (Figure 4.21).

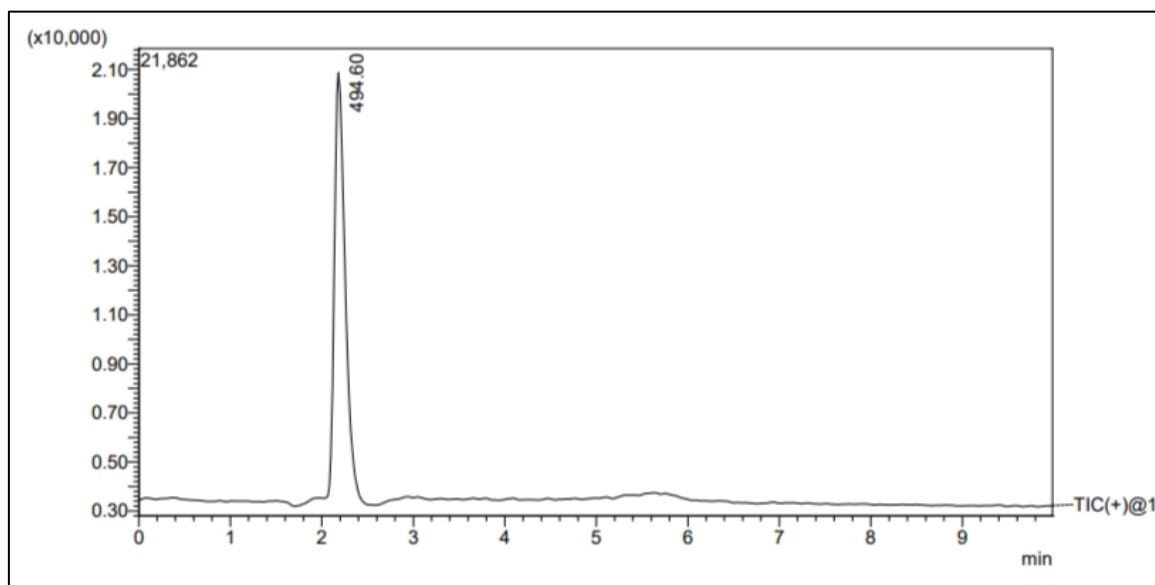


Figure 4.21: LC-MS of compound **78**

The functional group of compound **78** was determined from the FT-IR spectrum (Figure 4.22). The FT-IR shows absorption bands at 3410.91 cm^{-1} , 2927.96 cm^{-1} , 1644.50 cm^{-1} , 1463.27 cm^{-1} , and 1072.25 cm^{-1} which indicate the presence of OH group, C-H stretch, carbonyl group, aromatic C=C and C-O stretch, respectively. Besides that, the UV spectrum gave the λ_{max} at 225, 260 and 315 nm, suggesting the characteristics of xanthone.

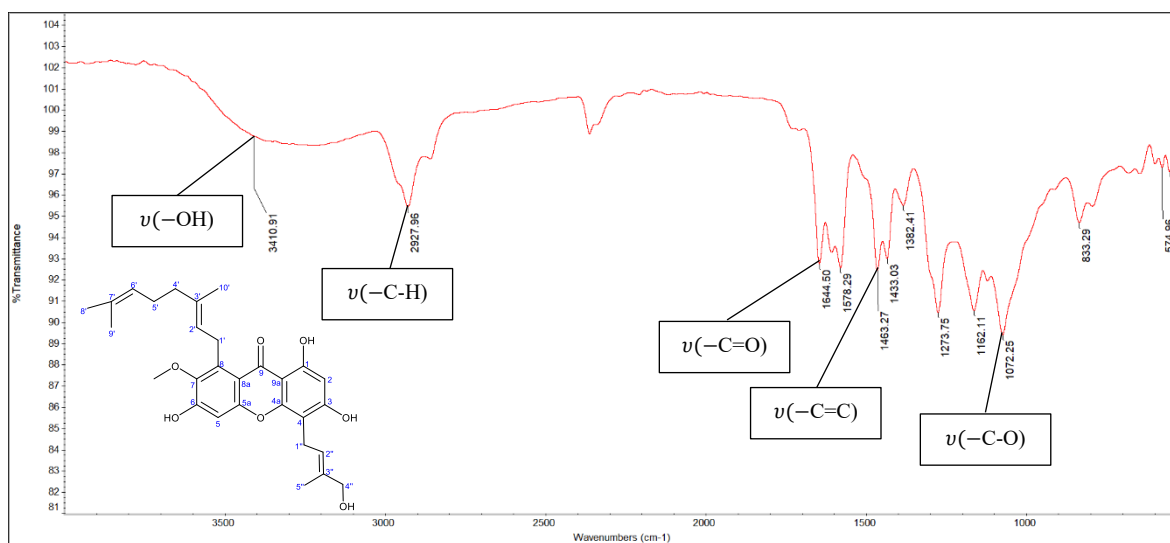


Figure 4.22: FT-IR of compound 78

Structure **78** was established by comparing its NMR spectral data (Figure 4.24, Figure 4.25, Table 4.4, and Appendix C). The ^1H NMR data of **78** revealed that there are two singlet aromatic protons (δ 6.26, 1H, s, H-2; δ 6.87, 1H, s, H-5), a chelated hydroxyl proton (δ 13.40, 1H, s, 1-OH), and one methoxy group [δ 3.80 (3H, s, 7-O-CH₃)]. In addition, one 2-methylpent-2-en-1-ol moiety and one geranyl unit were also present in the xanthone skeleton based on 1D and 2D NMR spectral data.

The sharp singlet at the lower field region, δ 13.40 (1-OH) attached to C-1, was confirmed by the cross-peaks of the ^2J to ^3J correlation of 1-OH with δ 161.6 (C-1), δ 98.8 (C-2), δ 103.7 (C-9a) in the HMBC spectra (Figure 4.23). Meanwhile, the methoxy moiety in compound **78** was detected from the resonance signal of CH₃ (δ 3.80, δ 62.0) crossed with the signal of δ 142.8 (C-7) in the HMBC spectrum suggesting its position at C-7.

A group of signals consists of a triplet at δ 5.42 (2H, $J=7.8$, H-2''), a doublet at δ 3.57 (2H, $J=7.8$, H-1''), along with two singlets at δ 1.78 (H-5''), δ 4.39 (H-4'') shows the presence of 2-methylpent-2-en-1-ol moiety in the structure. H-4'' shows a broad singlet, indicating it is a hydroxymethyl group (Taher et al., 2007). H-1'' and H-2'' were deduced to be adjacent to each other based on the ^1H - ^1H COSY spectrum with coupling constant value of 7.8 Hz. This unit was located on C-4 of the xanthonoid skeleton due to the proton H-1'', were found to have ^2J correlation with the quaternary carbon δ 103.4 (C-4) and δ 126.7 (C-2'') as well as ^3J correlation with δ 133.5 (C-3'').

The presence of geranyl substituent was confirmed with ^1H NMR the signal of two olefinic methine group [δ 5.26 (1H, t, H-2'), δ 5.02 (t, H-6')], three methyl [δ 1.54 (3H, s, H-8'), δ 1.59 (3H, s, H-9'), δ 1.82 (3H, s, H-10')] and three methylene [δ 4.08 (2H, d, H-1'), δ 2.02-2.04 (4H, m, H-4', H-5')]. The cross-peak of the geranyl unit (H-1') with

C-8 (δ 137.2), C-7 (δ 142.8), and C-8a (δ 111.9) in ^2J and ^3J correlation allowed it to locate at C-8 of the xanthone skeleton.

^{13}C NMR and DEPT spectra revealed the presence of a total of 29 carbons signals, including one downfield carbonyl carbon at δ 182.1 and 14 quaternary carbons, of which seven are oxygenated carbon (δ 161.6, 161.5, 153.9, 154.7, 155.7, 142.8 and 182.1). Meanwhile, the non-oxygenated quaternary carbons are resonated at δ 103.4, 137.2, 111.9, 103.7, 135.6 and 131.3. Besides, the ^{13}C NMR and DEPT spectra showed five methine carbon signals, five methylene signals and five methyl carbon signals. The HSQC spectrum affirmed the correlation of protonated carbons through ^1J correlations.

Based on all of the above spectral data, this compound has been identified as isocowanol (**78**) which has been also previously isolated by Taher et al. (2007).

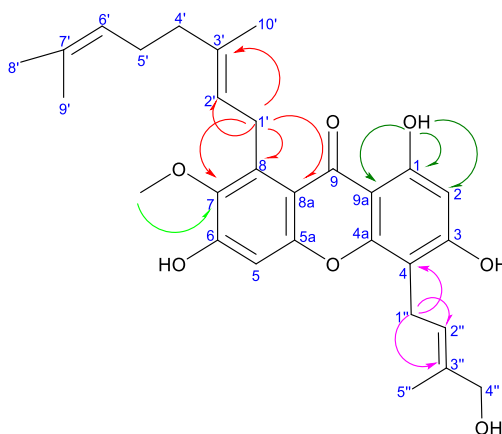


Figure 4.23: HMBC correlation in compound **78**

Table 4.4: 1D and 2D-NMR assignments of isocowanol (**78**)

Position	¹ H NMR	¹ H NMR*	¹³ C NMR	DEPT	HMBC
1-OH	13.40, <i>s</i> , 1H	13.41, <i>s</i> , 1H	161.6	C	C-2, C-9a, C-1
2	6.26, <i>s</i> , 1H	6.25, <i>s</i> , 1H	98.8	CH	C-4, C-9a, C-1, C-3
3			161.5	C	
4			103.4	C	
4a			153.9	C	
5	6.87, <i>s</i> , 1H	6.95, <i>s</i> , 1H	101.6	CH	C-8a, C-7, C-5a, C-6
5a			154.7	C	
6			155.7	C	
7			142.8	C	
7-O-CH ₃	3.80, <i>s</i> , 3H	3.80, <i>s</i> , 3H	62.0	CH ₃	C-7
8			137.2	C	
8a			111.9	C	
9			182.1	C	
9a			103.7	C	
1'	4.08, <i>d</i> , <i>J</i> =5.9 Hz, 2H	4.12, <i>d</i> , 2H	26.5	CH ₂	C-8a, C-2', C-3', C-8, C-7
2'	5.26, <i>t</i> , <i>J</i> =5.8, 6.0 Hz, 1H	5.31, <i>t</i> , 2H	123.2	CH	C-10', C-4'
3'			135.6	C	
4'	2.02, <i>m</i> , 2H	1.9-2.2, <i>m</i> , 4H	39.7	CH ₂	
5'	2.04, <i>m</i> , 2H		26.6	CH ₂	
6'	5.02, <i>t</i> , <i>J</i> =6.3, 6.4 Hz, 1H	5.02, <i>m</i> , 1H	124.3	CH	
7'			131.3	C	
8'	1.54, <i>s</i> , 3H	1.57, 3H	17.7	CH ₃	C-9', C-6', C-7'
9'	1.59, <i>s</i> , 3H	1.57, 3H	25.6	CH ₃	C-8', C-6', C-7'
10'	1.82, <i>s</i> , 3H	1.83, 3H	16.5	CH ₃	C-4', C-2', C-3'
1''	3.57, <i>d</i> , <i>J</i> =7.8 Hz, 2H	3.55, <i>d</i> , 2H	21.7	CH ₂	C-4, C-2'', C-3''
2''	5.42, <i>t</i> , <i>J</i> =7.8, 7.9 Hz, 1H	5.31, <i>d</i> , 2H	126.7	CH	C-5'', C-4''
3''			133.5	C	
4''	4.39, <i>s</i> , 2H	4.40, <i>s</i> , 2H	62.7	CH ₂	C-2'', C-3''
5''	1.78, <i>s</i> , 3H	1.76, 3H	22.7	CH ₃	C-4'', C-2'', C-3''

¹H NMR* references adapted from Taher et al. (2007)

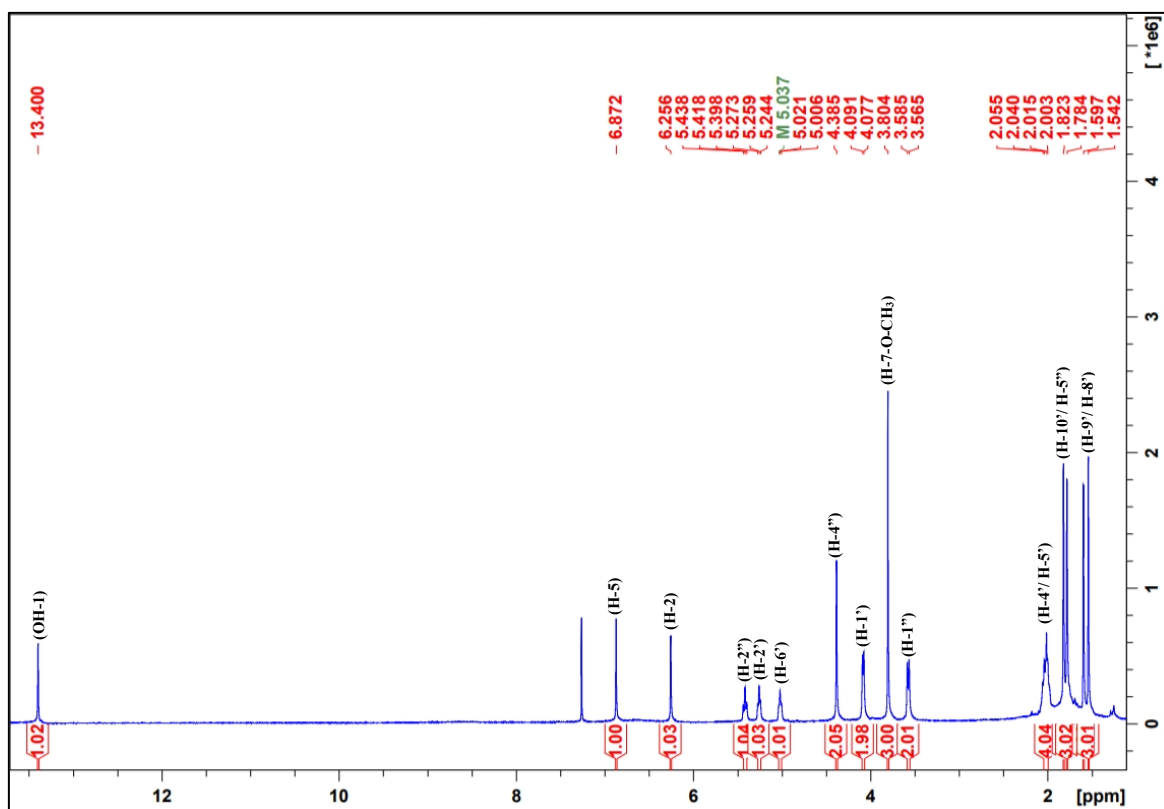


Figure 4.24: ¹H NMR of compound 78

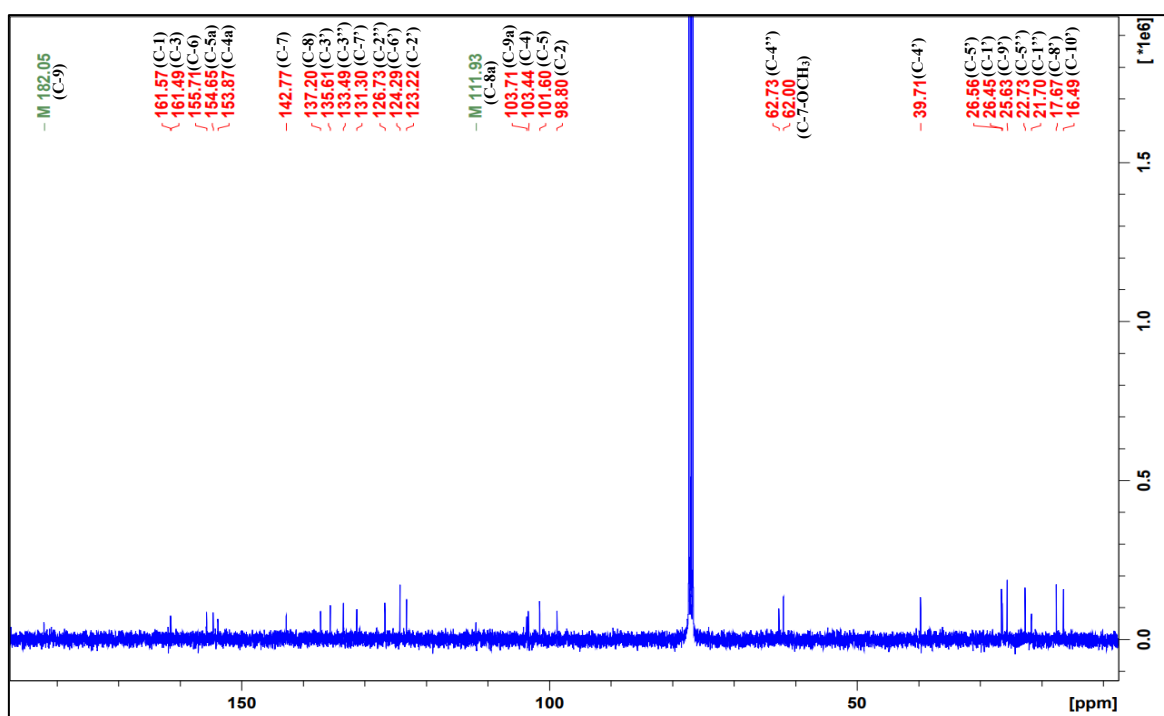


Figure 4.25: ¹³C NMR of compound 78

4.2.5 Characterization of 9-Hydroxycalabaxanthone (79)

9-Hydroxycalabaxanthone (**79**) was obtained as orange oil. A molecular formula of $C_{24}H_{24}O_6$ was determined from the LC-MS spectra, corresponding to the structure with m/z of 408.45.

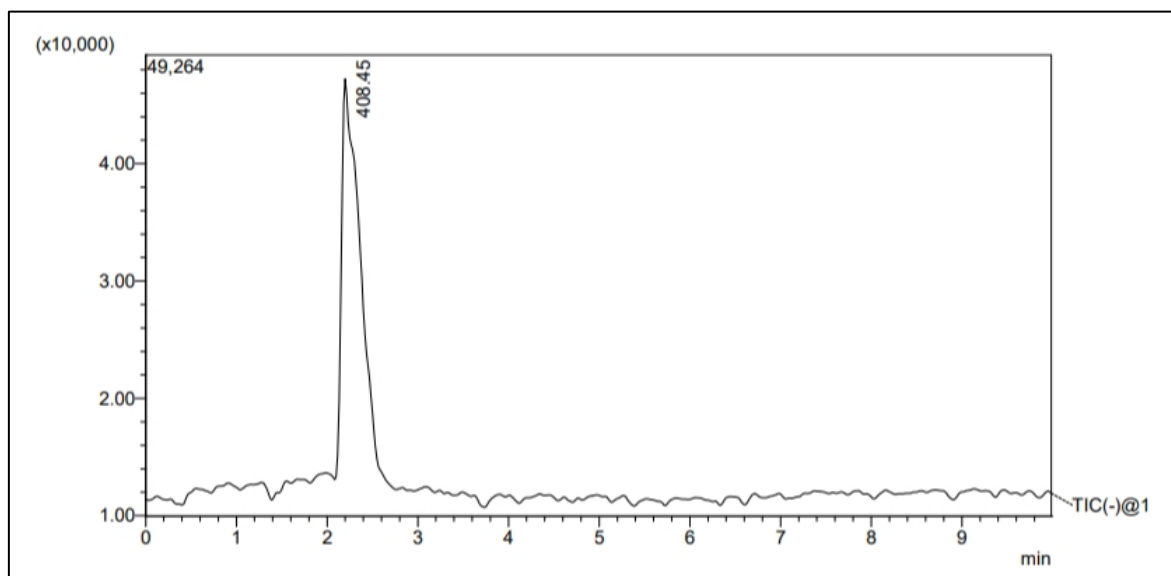


Figure 4.26: LC-MS of compound **79**

The FT-IR spectrum exhibited bands for OH stretch (3426.71 cm^{-1}), C-H stretch (2922.92 cm^{-1}), C=O stretch (1643.85 cm^{-1}), and C=C stretch (1459.26 cm^{-1}). Meanwhile, the UV spectrum gave the λ_{max} at 285 nm.

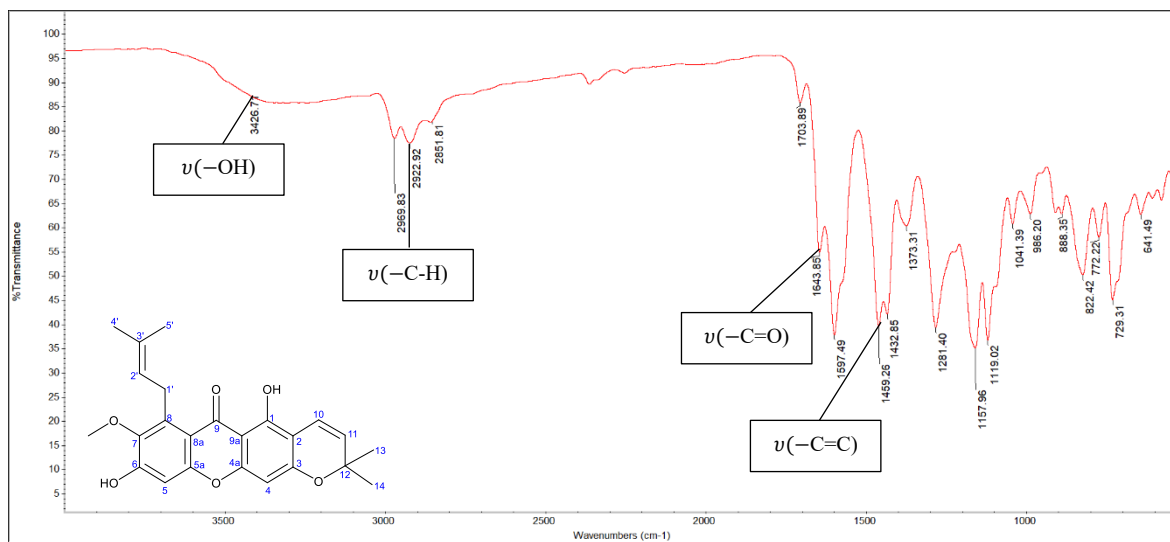


Figure 4.27: FT-IR of compound 79

The ^1H NMR spectrum displayed a singlet at δ 13.72 for deshielded phenolic hydroxyl group. It appeared at the deshielded region due to the presence of electron-drawing groups, such as carbonyls, next to the hydroxyl group can further reduce electron density around the hydroxyl hydrogen (Mitschke et al., 2023). The positioning of the chelated hydroxyl group at C-1 was confirmed by the HMBC cross-peaks of 1-OH with δ 157.9 (C-1), δ 104.5 (C-2), δ 103.7 (C-9a) in ^2J to ^3J correlation. The presence of pyrano moiety was observed at δ 6.75 (H-10), δ 5.59 (H-11), δ 1.48 (H-13 and H-14). The COSY-NMR spectrum and a similar coupling constant of 10.0 Hz confirmed a pair of adjacent vinylic protons (Daud et al., 2021). The pyrano moiety was found to be fused to position C-2 and C-3 by referring to the HMBC correlation contours. The HMBC spectrum showed a ^3J correlation between the proton δ 6.75 (H-10) and the aromatic oxygenated carbon at δ 159.8 (C-3) and δ 157.9 (C-1). Meanwhile, a ^2J correlation was found between δ 6.75 (H-10) and carbon at δ 104.5 (C-2). The proton δ 5.59 (H-11) showed a ^3J correlation with δ 104.5 (C-2).

The ^1H NMR spectrum also revealed a presence of methoxy group at δ 3.82 (3H, s, 7-O-CH₃). In the HMBC spectrum, the resonance signal of CH₃ (δ 3.82, δ 61.9) crossed with the signal of δ 142.7 (C-7), indicating methylation of the OH at the C-7 position.

The proton signals at δ 5.27 (1H, $J=7.0, 7.1$, H-2'), δ 4.09 (2H, $J=6.9$, H-1') as well as δ 1.84 (3H, H-4') and δ 1.71 (3H, H-5') indicate the presence of prenyl groups in the structure. The attachment of the prenyl group in the structure was supported by a long-range coupling between the protons and carbons *via* HMBC analysis (Wang et al., 2018). The ^2J to ^3J correlation of δ 4.09 (H-1') with carbon δ 137.0 (C-8), δ 142.7 (C-7), δ 112.1 (C-8a) prove the attachment of prenyl group at C-8.

After analysing all the spectral data (Figure 4.26, Figure 4.27, Figure 4.29, Figure 4.30, Table 4.5, and Appendix D), compound **79** was identified as 9-hydroxycalabaxanthone (**79**). The HMBC correlation of the compound is shown in Figure 4.28. All the data is consistent with Wang et al. (2018).

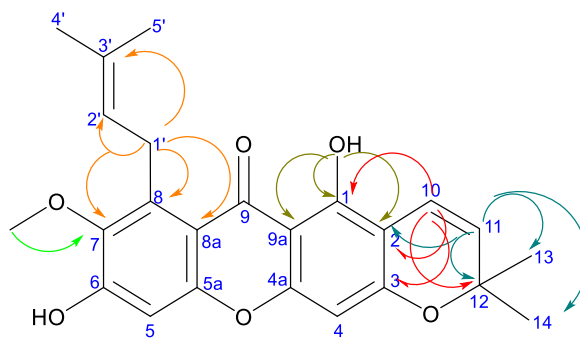


Figure 4.28: HMBC correlation in compound **79**

Table 4.5: 1D and 2D-NMR assignments of 9-hydroxycalabaxanthone (**79**)

Position	¹ H NMR	¹³ C NMR	¹³ C NMR*	DEPT	HMBC
1	13.72, <i>s</i> , 1H	157.9	157.8	C	C-9a, C-2, C-1
2		104.5	104.3	C	
3		159.8	159.8	C	
4	6.24, <i>s</i> , 1H	94.2	94.0	CH	C-9a, C-2, C-4a, C-3
4a		156.2	156.1	C	
5	6.83, <i>s</i> , 1H	101.8	101.6	CH	C-8a, C-7, C-6, C-5a, C-9
5a		155.7	155.6	C	
6		154.7	154.5	C	
7		142.7	142.7	C	
7-O-CH ₃	3.82, <i>s</i> , 3H	61.9	61.8	CH ₃	C-7
8		137.0	137.9	C	
8a		112.1	112.1	C	
9		181.9	181.8	C	
9a		103.7	103.6	C	
10	6.75, <i>d</i> , <i>J</i> =10.0 Hz, 1H	115.7	115.6	CH	C-12, C-2, C-1, C-3
11	5.59, <i>d</i> , <i>J</i> =10.0 Hz, 1H	127.2	126.9	CH	C-13/14, C-12, C-2
12		77.9	77.8	C	
13 & 14	1.48, <i>s</i> , 6H	28.3	28.3	CH ₃	C-13/14, C-12, C-10, C-11, C-3
1'	4.09, <i>d</i> , <i>J</i> =6.2 Hz, 2H	26.5	26.5	CH ₂	C-8a, C-2', C-3', C-8, C-7
2'	5.27, <i>t</i> , <i>J</i> =5.0 Hz, 1H	123.2	123.1	CH	C-4', C-5'
3'		132.2	131.8	C	
4'	1.84, <i>s</i> , 3H	18.2	18.1	CH ₃	C-5', C-2', C-3'
5'	1.71, <i>s</i> , 3H	25.8	25.6	CH ₃	C-4', C-2', C-3'

¹³C NMR*references adapted from Wang et al. (2018)

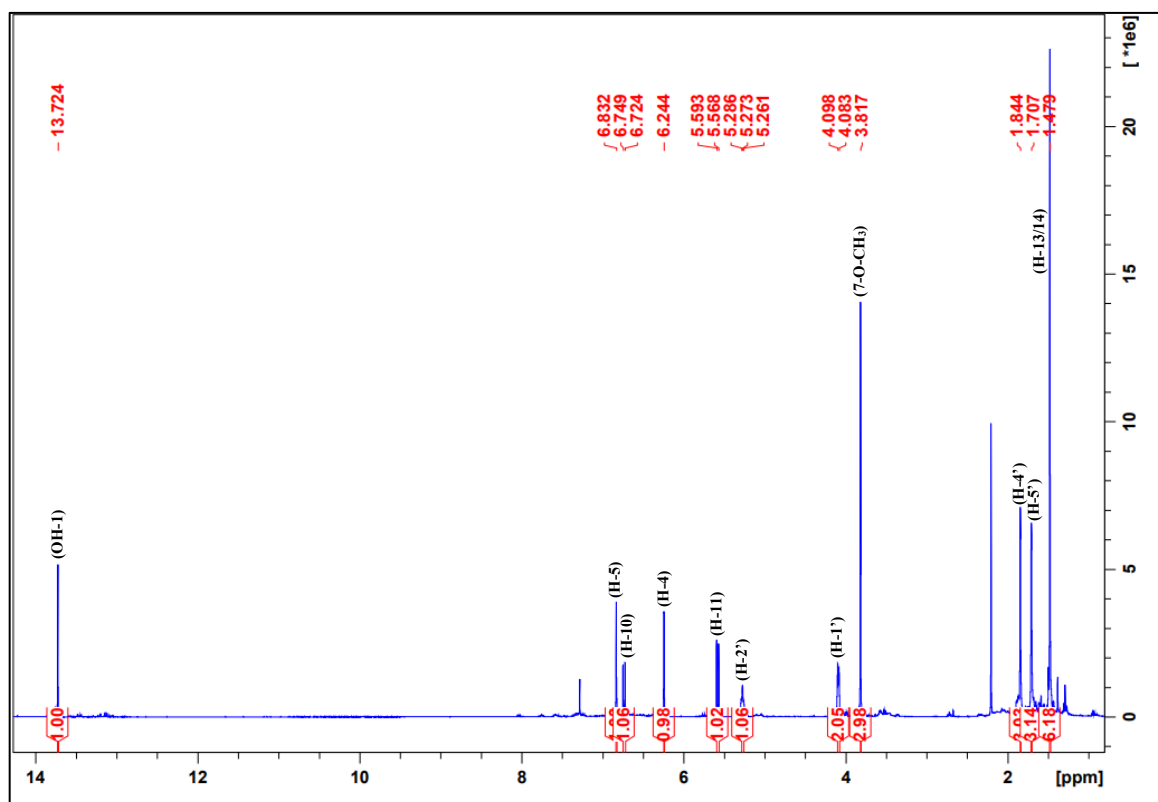


Figure 4.29: ^1H NMR of compound 79

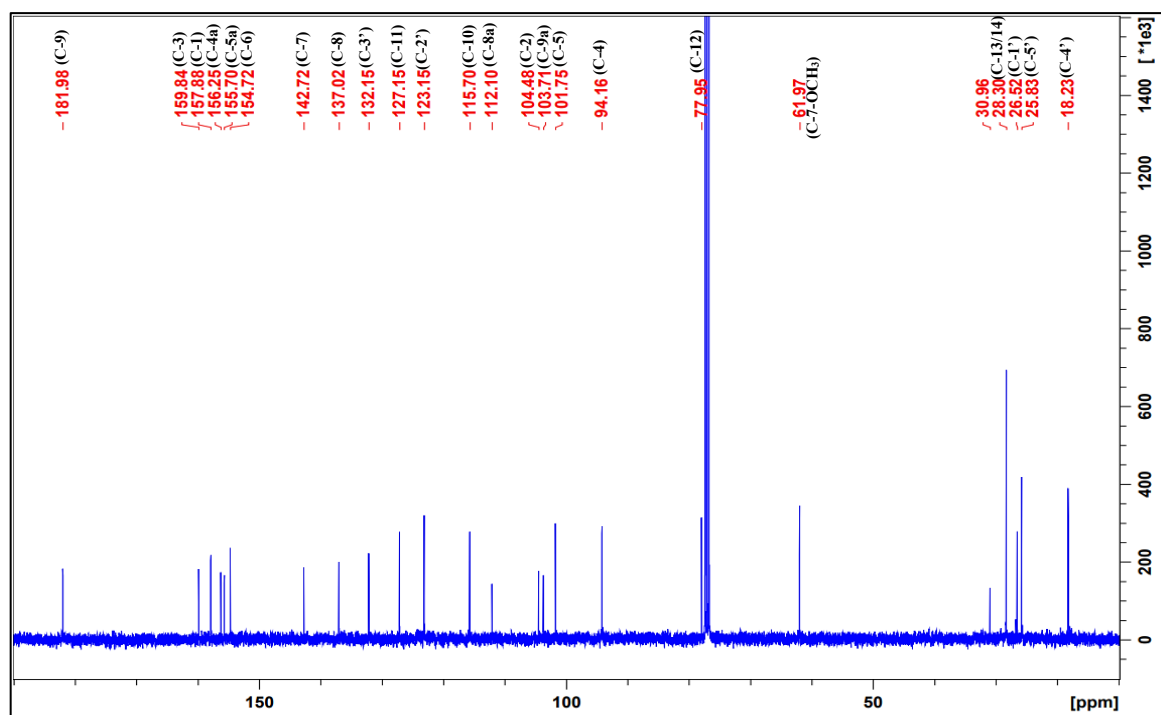


Figure 4.30: ^{13}C NMR of compound 79

4.2.6 Characterization of Stigmasterol (**59**)

Stigmasterol (**59**) was isolated as a white needle crystal with a melting point of 175 °C (Lit 174-176 °C) (Chaturvedula & Prakash, 2012). The compound has a molecular formula of $C_{29}H_{48}O$, as evidenced by an LC-MS spectrum that revealed a molecular peak at m/z 412.70, as shown in Figure 4.31.

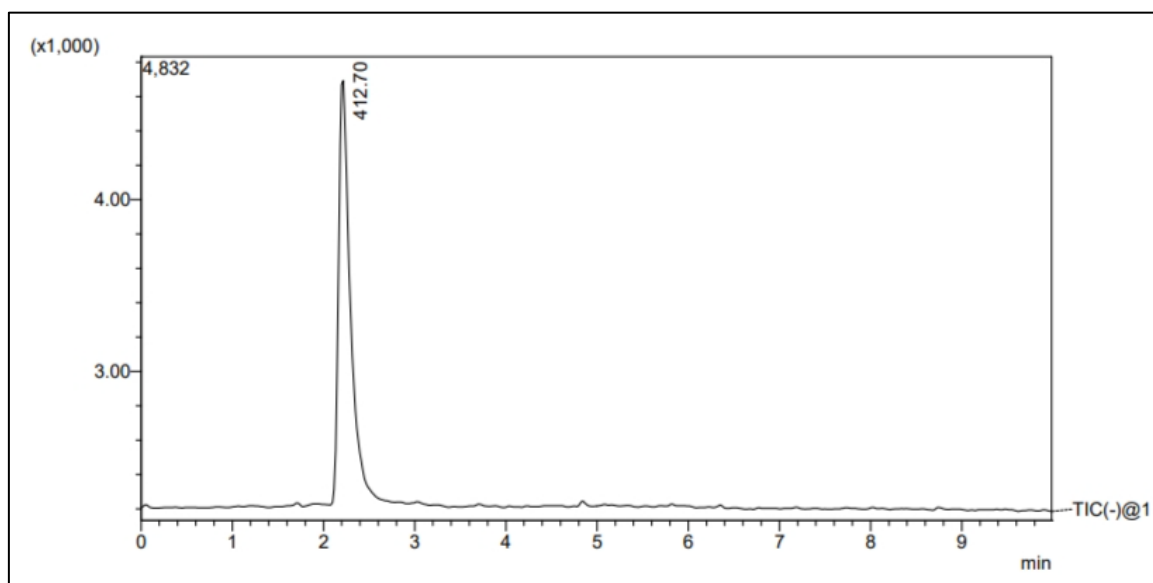


Figure 4.31: LC-MS of compound **59**

The functional group of **59** was determined from the FT-IR spectrum (Figure 4.32). The values are 3415.41 cm^{-1} (OH group), 2930.93 cm^{-1} (C-H stretch), 1456.09 cm^{-1} (C=C stretch), and 1049.79 cm^{-1} (C-O stretch). Conversely, the UV spectrum revealed λ_{max} 203 nm, indicating the presence of conjugated double bonds on aromatic rings in the structure (Zhang et al., 2024).

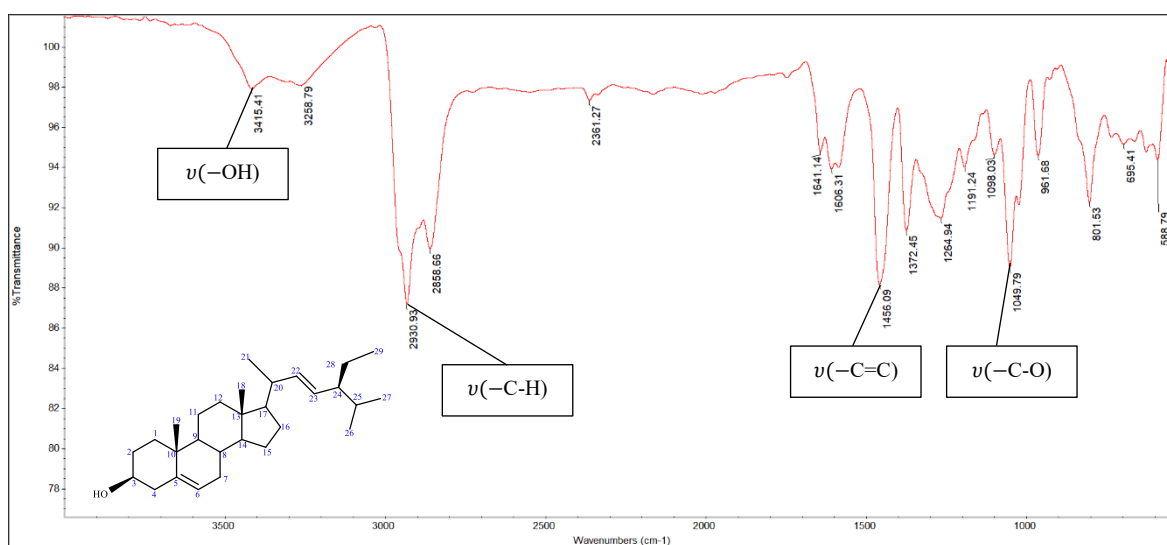
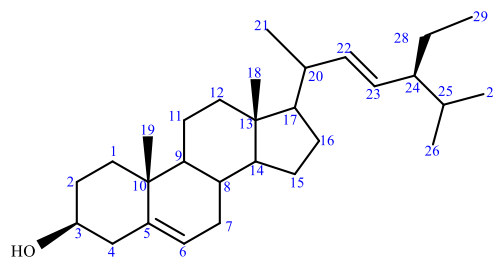


Figure 4.32: FT-IR of compound **59**

^1H NMR (CDCl_3 , 500 MHz) spectrum displayed the presence of two methyl singlets (δ 0.69 and δ 1.00), and four methyl in the range δ 0.79-0.83 with the integration of 12 protons. It also showed proton H-3 as a multiplet at δ 3.50, which commonly found in sterol moiety (Chaturvedula & Prakash, 2012). The olefinic protons of compound **59** appeared at δ 4.99, 5.13, and 5.33. For ^{13}C NMR, the chemical shift of alkene carbon in compound **59** was observed at δ 140.8 (C-5), 138.4 (C-22), 129.4 (C-23), and 121.8 (C-6). Based on a comparison with ^1H and ^{13}C NMR, it was following the isolated stigmasterol by Kamal et al. (2016). Figures 4.33, Figure 4.34, and Table 4.6 below display the ^1H and ^{13}C NMR spectra, as well as the summarised data.

Table 4.6: ^1H NMR and ^{13}C NMR assignments of stigmasterol (**59**)



Position	^1H NMR	^{13}C NMR	^{13}C NMR *
1		37.3	37.3
2		31.7	31.7
3-OH	3.50, <i>m</i> , 1H	71.9	71.9
4		42.3	42.3
5		140.8	140.8
6	5.33, <i>t</i> , $J=2.6$ Hz, 1H	121.8	121.8
7		31.9	32.1
8		31.9	32.0
9		50.2	50.2
10		36.6	36.6
11		21.2	21.2
12		39.8	39.8
13		42.4	42.4
14		56.9	56.8
15		24.4	24.4
16		28.9	28.3
17		56.0	56.1
18	1.00, <i>s</i> , 3H	12.3	11.9
19	0.79-0.83, <i>m</i> , 3H	19.1	19.1
20		40.6	40.6
21	0.79-0.83, <i>m</i> , 3H	21.3	20.0
22	4.99, <i>m</i> , 1H	138.4	138.4
23	5.13, <i>m</i> , 1H	129.4	129.2
24		51.3	51.3
25		45.9	34.0
26	0.79-0.83, <i>m</i> , 3H	19.5	18.9
27	0.79-0.83, <i>m</i> , 3H	21.3	21.3
28		25.5	25.5
29	0.69, <i>s</i> , 3H	12.1	12.1

^{13}C NMR * references adapted from Kamal et al. (2016)

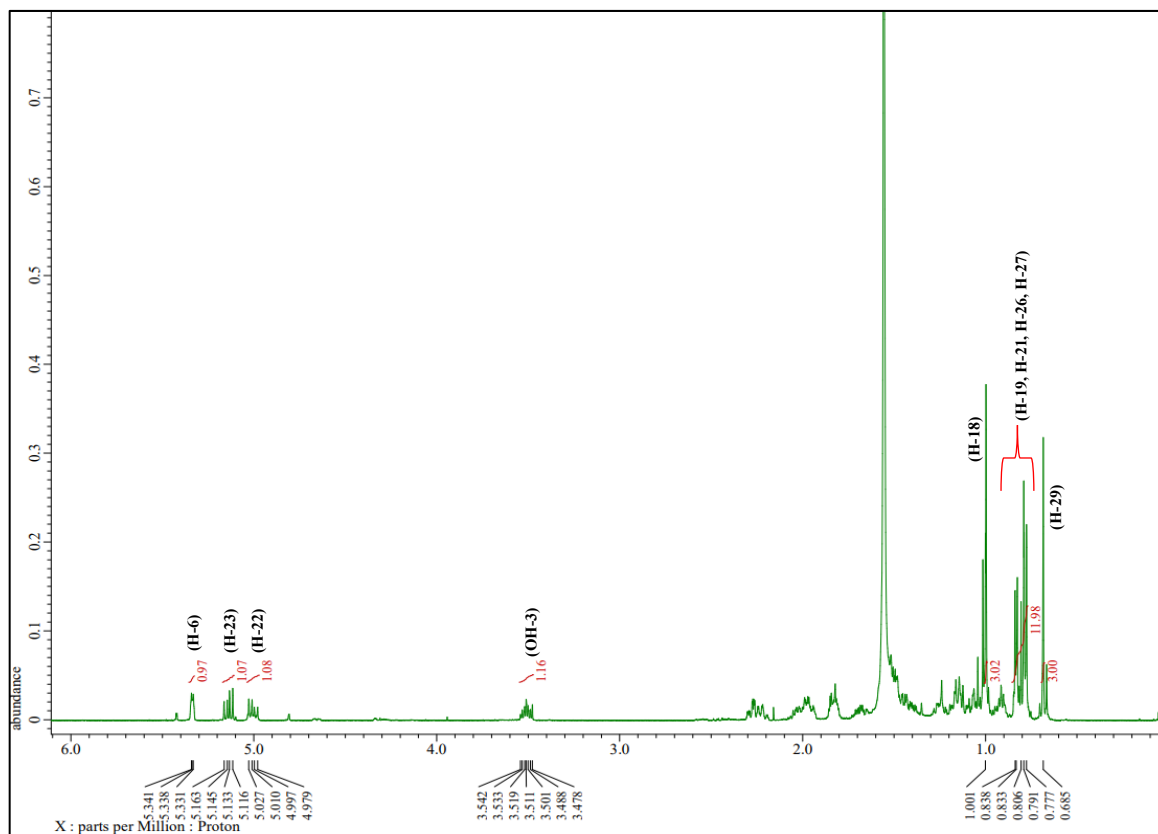


Figure 4.33: ¹H NMR of compound **59**

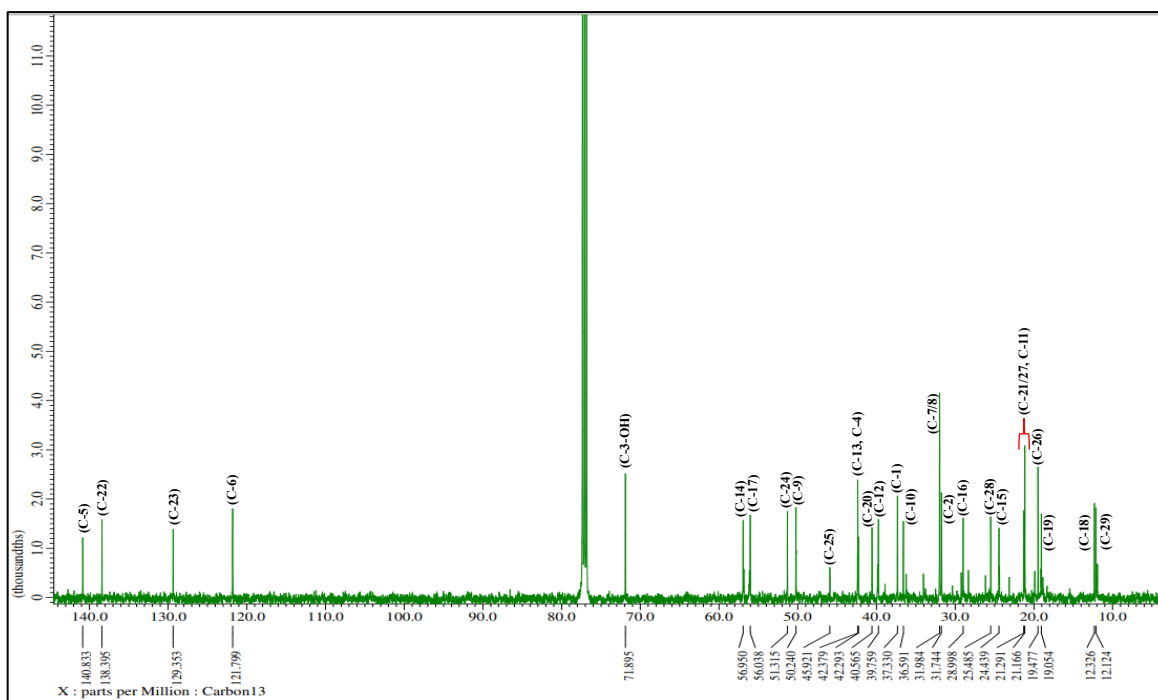


Figure 4.34: ¹³C NMR of compound **59**

4.3 Structural Modification of Rubraxanthone (2)

The structural modification was successfully carried out on the major compound isolated from *G. dryobalanoides*, rubraxanthone (**2**) with the envision of improved biological properties by adding an alkyl chain for lipophilicity apart from pronounced literature precedence stating an alkyl chain imitated structural resemblance to the bacteria cell wall hence improved mobility for penetration (Gabriel et al., 2007). Notably, a phenolic hydroxyl group in rubraxanthone (**2**) enables the formation of ethers *via* Williamson etherification. This reaction, characterized by an S_N2 mechanism, involves the deprotonation of the hydroxyl group to form an alkoxide ion, which subsequently reacts with a primary alkyl halide to introduce an alkyl substituent (Abd Halim et al., 2023). In this study, the semi-synthesis of compound **2** yielded five new *O*-alkylation-rubraxanthones, namely, 3-*O*-propyl-rubraxanthone (**80**), 6-*O*-propyl-rubraxanthone (**81**), 3,6-*O*-pentyl-rubraxanthone (**82**), 3,6-*O*-hexyl-rubraxanthone (**83**) and 6-*O*-butyl-rubraxanthone (**84**). All of the newly semi-synthesized compounds selectively alkylated at the C-3 and C-6 positions but not C-1 due to the less reactivity and its ability to form an intramolecular hydrogen bond with the C-9 carbonyl group (Zou et al., 2013).

4.3.1 Characterization of 3-*O*-propyl-rubraxanthone (**80**) and 6-*O*-propyl-rubraxanthone (**81**)

Semi-synthesis of compound **2** with 1-bromopropane afforded two products, 3-*O*-propyl-rubraxanthone (**80**) and 6-*O*-propyl-rubraxanthone (**81**). Compound **80** was obtained as a bright yellow powder with a melting point of 214 °C. Meanwhile, compound **81** was obtained as a pale-yellow powder with a melting point of 215 °C. Both compounds **80** and **81** have a molecular formula of C₂₇H₃₂O₆ as supported by LC-MS spectrum which showed a molecular peak at m/z 452.20 (Figure 4.35 and Figure 4.36). For the UV λ_{max}

absorptions, compound **80** was observed to have a peak at 255 and 310 nm, while compound **81** had a peak at 250 and 310 nm. Both indicate a xanthone skeletal structure, where the chromophore of 255 and 250 nm ascribed to the presence of $\pi\text{-}\pi^*$ electron excitation (-C=C- , aromatic ring) and 310 nm ascribed the presence of $\text{n-}\pi^*$ electron excitation (-C=C-C=O , conjugated carbonyl group) (Ngoupayo et al., 2009).

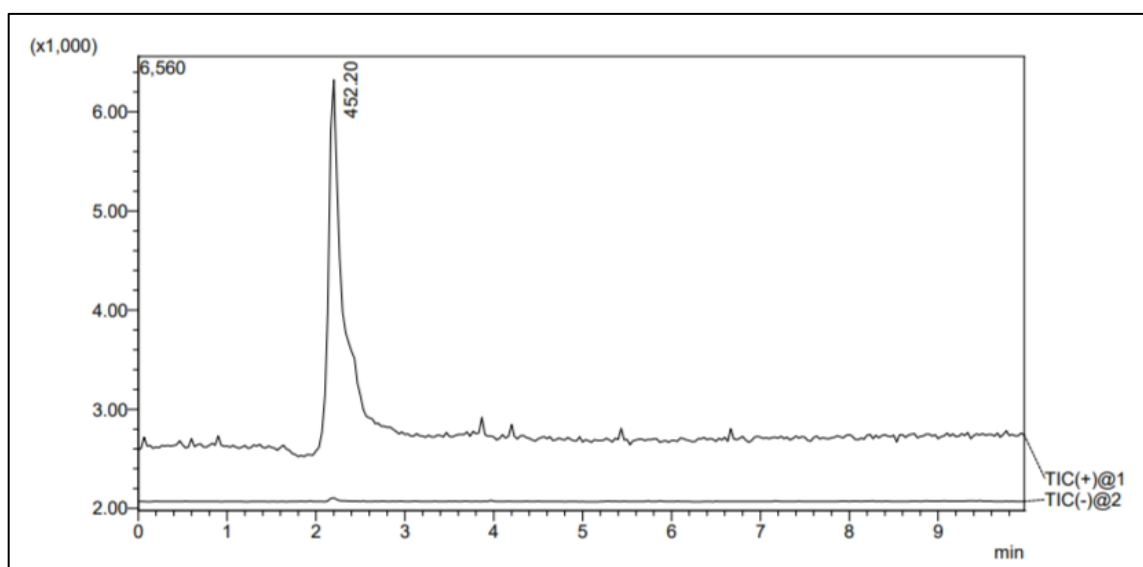


Figure 4.35: LC-MS of compound **80**

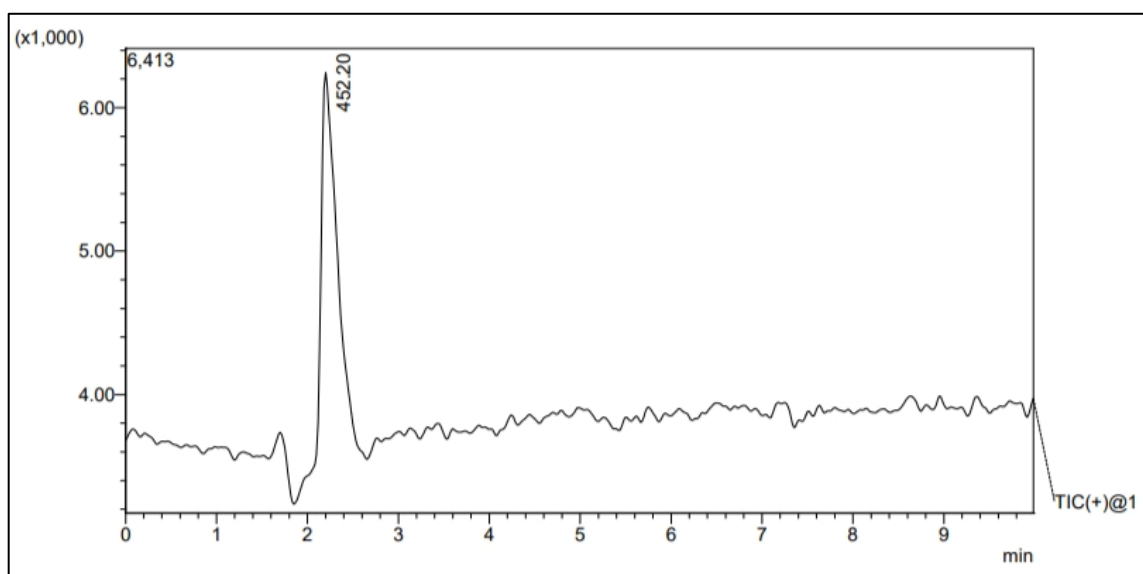


Figure 4.36: LC-MS of compound **81**

The functional group of both synthesized compounds was determined from the FT-IR spectrum (Figure 4.37 and Figure 4.38). The FT-IR spectrum for compound **80** displayed absorptions of OH stretch (3393.13 cm^{-1}), C-H stretch (2925.16 cm^{-1}), C=O group (1646.22 cm^{-1}), aromatic C=C (1463.71 cm^{-1}) and C-O stretch (1084.54 cm^{-1}). Compound **81**, on the other hand, exhibited bands for OH stretch (3327.40 cm^{-1}), C-H stretch (2922.17 cm^{-1}), C=O stretch (1651.48 cm^{-1}), C=C stretch (1463.06 cm^{-1}) and C-O stretch (1081.02 cm^{-1}). As can be observed from the FT-IR spectra, there was a slightly different between the OH peak in compound **80** and **81**, where the OH stretch were for compound **80** had appeared in the higher frequency. This is due to steric hindrance can limit the ability of the OH group to form hydrogen bonds with other molecules, including other OH groups in the same molecule. This reduced interaction can cause higher energy vibrations, which appear at higher wavenumbers (Asprion et al., 2001).

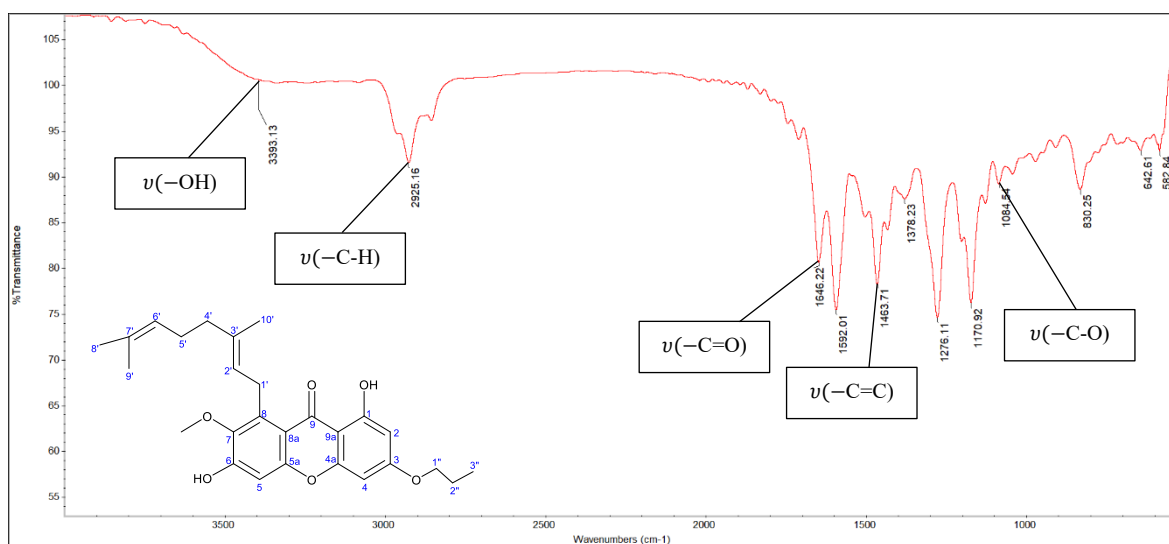


Figure 4.37: FT-IR of compound 80

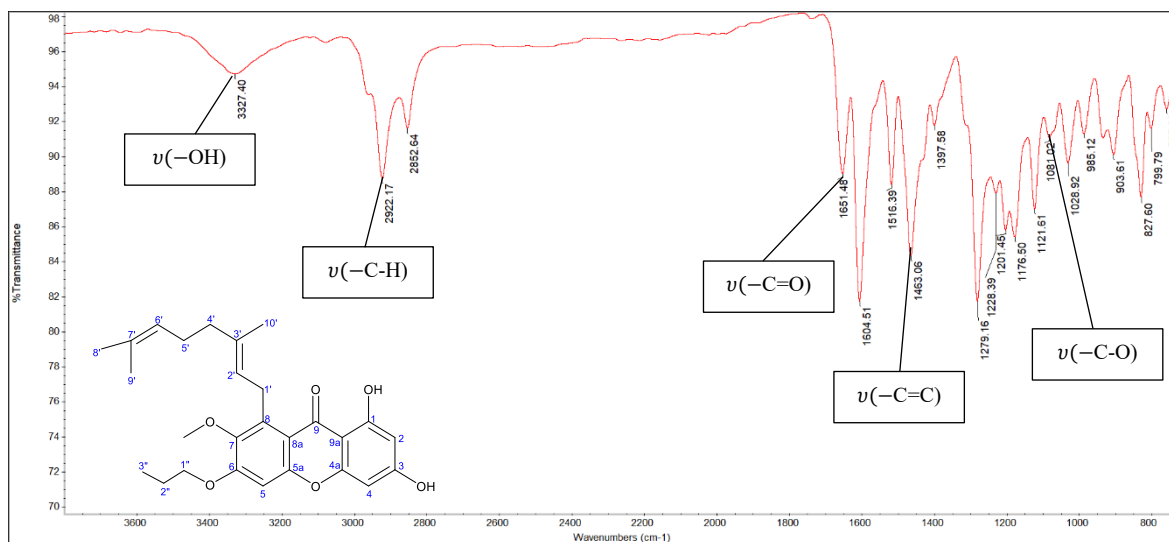


Figure 4.38: FT-IR of compound **81**

The NMR data showed similar substitution patterns of compound **2** with some additional peaks at the ^1H and ^{13}C NMR spectra. From the ^1H NMR of compound **80** (Figure 4.40), two additional triplets revealed a new *O*-alkylated group at δ 4.10 (2H, t, H-1'') and a methyl group at δ 1.06 (3H, t, H-3''). Meanwhile, the additional peak for compound **81** (Figure 4.44) was displayed at δ 4.20 (2H, t, H-1'') and δ 1.13 (3H, t, H-3''). The proton signals H-1'' for both compound **80** and **81** were deshielded compared to the proton signal H-3'' because their position is close to the electronegative atom "O", which can cause the electron-withdrawing inductive effect (Yuanita et al., 2023). From the ^{13}C NMR spectra (Figure 4.41 and Figure 4.45), the formation of *O*-alkylation of compounds **80** and **81** with rubraxanthone was proven due to the additional carbon peak at δ 69.9 (C-1'') and δ 70.4 (C-1''), respectively.

The attachment of the substituent in the structure was supported by a long-range coupling between the protons and carbons *via* HMBC analysis (Figure 4.42 and Figure 4.46). In the HMBC spectrum of compound **80**, δ 4.10 (H-1'') has ^3J correlations with δ 165.6

(C-3) deduced that the propane side chain was connected to C-3 (Figure 4.39). For compound **81**, δ 4.20 (H-1'') has ^3J correlations with δ 158.1 (C-6) deduced that the propane side chain was connected to C-6 (Figure 4.43).

The justification above concluded that the new semi-synthesized compound 3-*O*-propyl-rubraxanthone (**80**) and 6-*O*-propyl-rubraxanthone (**81**) were successfully synthesized from rubraxanthone (**2**). All the data are summarized in Table 4.7 and Table 4.8.

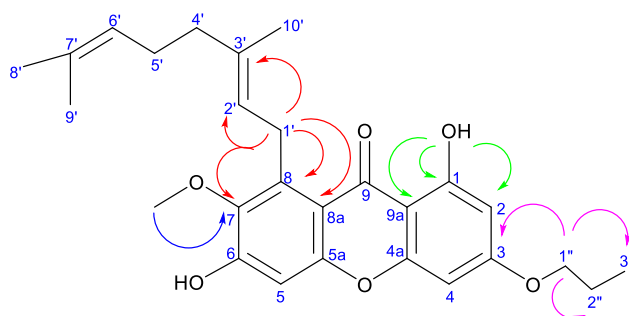


Figure 4.39: HMBC correlation in compound **80**

Table 4.7: 1D and 2D-NMR assignments of 3-*O*-propyl-rubraxanthone (**80**)

Position	¹ H NMR	¹³ C NMR	¹³ C NMR*	DEPT	HMBC
1-OH	13.47, <i>s</i> , 1H	163.7	163.9	C	C-2, C-9a, C-1
2	6.28, <i>d</i> , <i>J</i> =2.2 Hz, 1H	97.1	97.8	CH	C-4, C-9a
3		165.6	164.3	C	
4	6.42, <i>d</i> , <i>J</i> =2.3 Hz, 1H	91.9	92.9	CH	C-2, C-9a, C-4a, C-3
4a		156.9	156.9	C	
5	6.87, <i>s</i> , 1H	101.9	101.9	CH	C-8a, C-7, C-5a, C-6
5a		155.5	155.3	C	
6		156.9	156.4	C	
7		143.8	143.6	C	
7-O-CH ₃	3.82, <i>s</i> , 3H	60.5	60.5	CH ₃	C-7
8		137.4	137.2	C	
8a		111.1	111.1	C	
9		181.9	181.7	C	
9a		103.4	102.9	C	
1'	4.13, <i>d</i> , <i>J</i> =6.5 Hz, 2H	25.9	25.9	CH ₂	C-8a, C-2', C-3', C-8, C-7
2'	5.29, <i>t</i> , <i>J</i> =6.5, 6.6 Hz, 1H	123.8	124.0	CH	
3'		134.3	134.1	C	
4'	1.99, <i>m</i> , 2H	39.6	39.6	CH ₂	C-5', C-2', C-3'
5'	2.07, <i>m</i> , 2H	26.4	26.4	CH ₂	C-4'
6'	5.05, <i>t</i> , <i>J</i> =6.9, 7.0 Hz, 1H	124.3	124.3	CH	
7'		130.7	130.6	C	
8'	1.57, <i>s</i> , 3H	24.8	24.9	CH ₃	C-9', C-6', C-7'
9'	1.54, <i>s</i> , 3H	16.8	16.9	CH ₃	C-8', C-6', C-7'
10'	1.85, <i>s</i> , 3H	15.7	15.8	CH ₃	C-4', C-2', C-3'
1''	4.10, <i>t</i> , <i>J</i> =6.5 Hz, 2H	69.9		CH ₂	C-3'', C-2'', C-3
2''	1.83, <i>m</i> , 2H	22.1		CH ₂	C-3'', C-1''
3''	1.06, <i>t</i> , <i>J</i> =7.4 Hz, 3H	9.7		CH ₃	C-1'', C-2''

¹³C NMR* rubraxanthone (**2**)

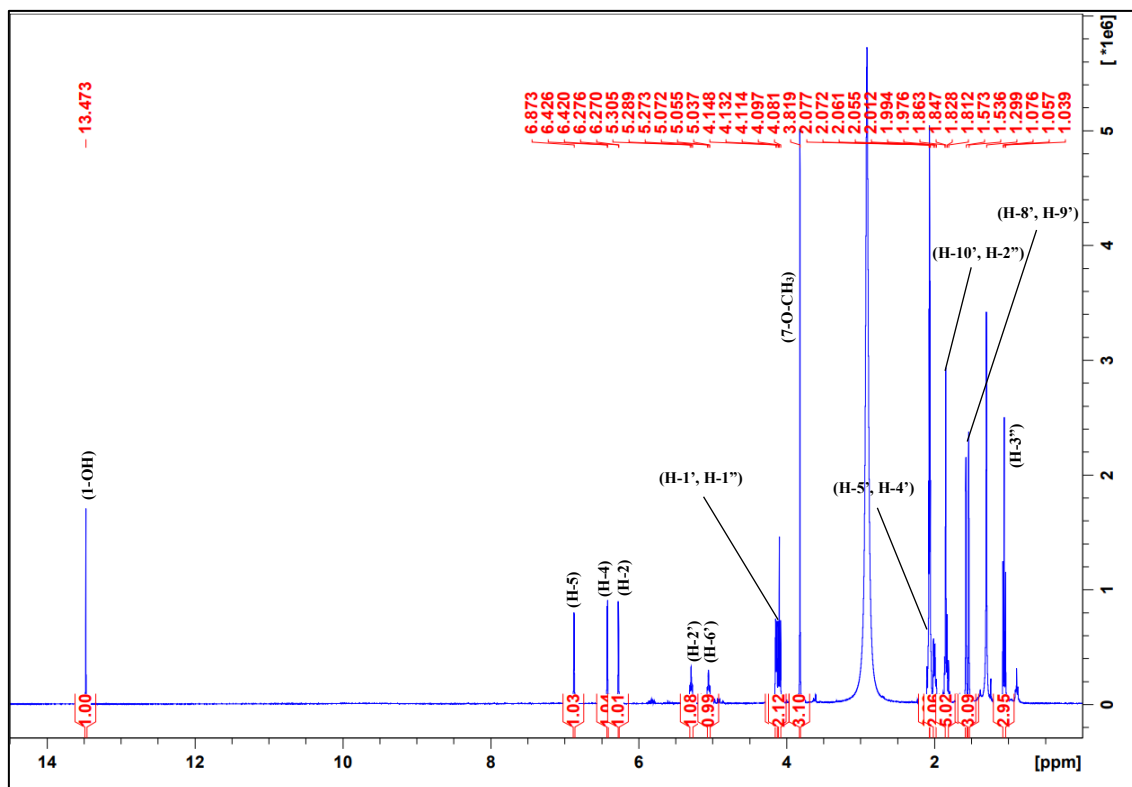


Figure 4.40: ¹H NMR of compound 80

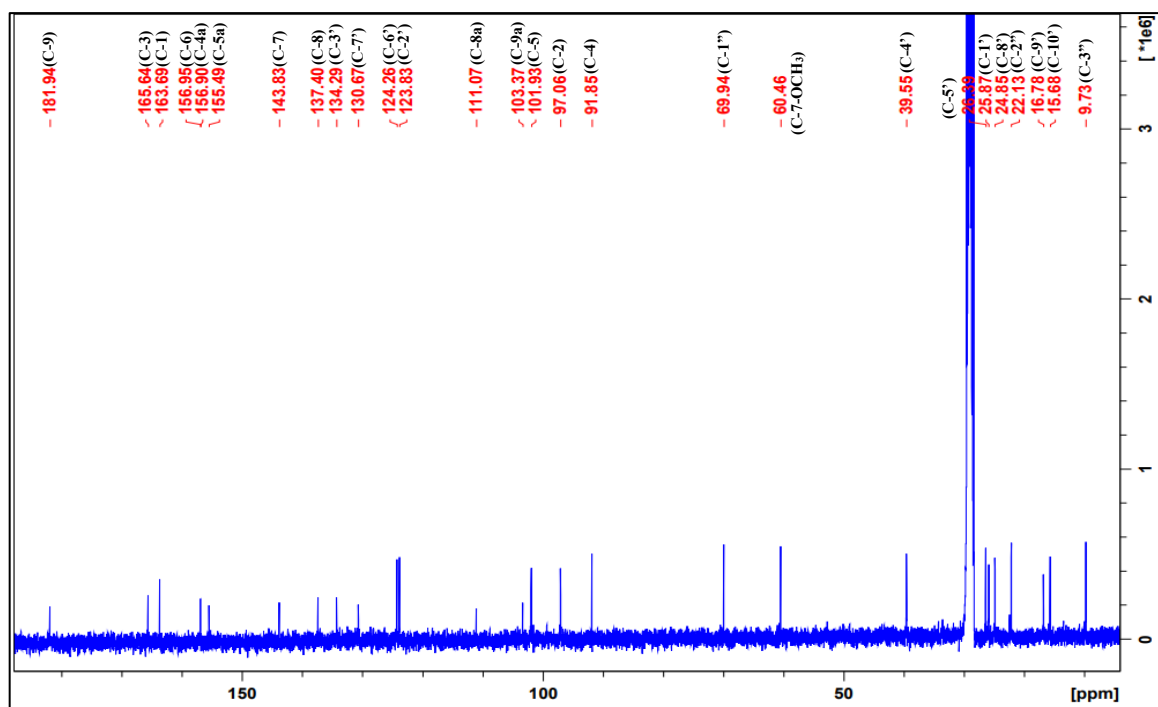


Figure 4.41: ¹³C NMR of compound 80

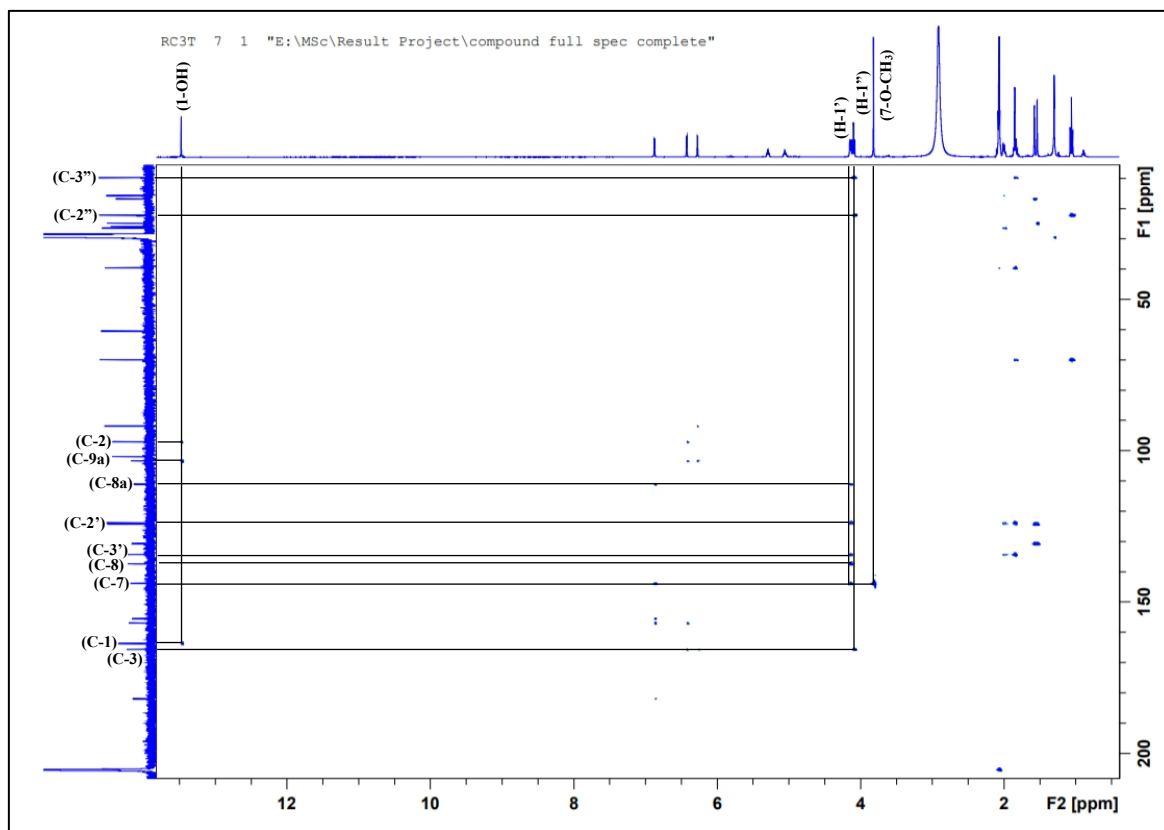


Figure 4.42: HMBC spectrum of compound **80**

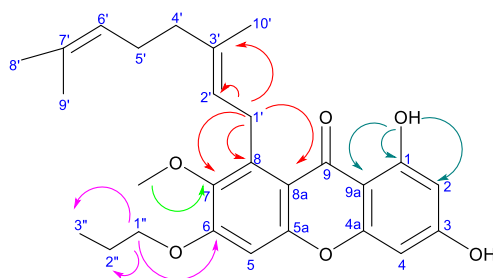


Figure 4.43: HMBC correlation in compound **81**

Table 4.8 1D and 2D-NMR assignments of 6-*O*-propyl-rubraxanthone (**81**)

Position	¹ H NMR	¹³ C NMR	¹³ C NMR*	DEPT	HMBC
1-OH	13.48, <i>s</i> , 1H	163.9	163.9	C	C-2, C-9a, C-1
2	6.22, <i>d</i> , <i>J</i> =2.1 Hz, 1H	97.9	97.8	CH	C-4, C-9a, C-1
3		164.7	164.3	C	
4	6.33, <i>d</i> , <i>J</i> =2.0 Hz, 1H	92.9	92.9	CH	C-2, C-9a, C-4a, C-3, C-9
4a		157.1	156.9	C	
5	6.98, <i>s</i> , 1H	99.2	101.9	CH	C-8a, C-8, C-7, C-5a, C-6, C-9
5a		155.4	155.3	C	
6		158.1	156.4	C	
7		144.4	143.6	C	
7-O-CH ₃	3.83, <i>s</i> , 3H	60.1	60.5	CH ₃	C-7
8		136.5	137.2	C	
8a		111.2	111.1	C	
9		181.9	181.7	C	
9a		102.9	102.9	C	
1'	4.16, <i>d</i> , <i>J</i> =6.6 Hz, 2H	25.7	25.9	CH ₂	C-8a, C-2', C-3', C-8, C-7
2'	5.27, <i>t</i> , <i>J</i> =6.6, 6.7 Hz, 1H	123.8	124.0	CH	C-10', C-4'
3'		134.2	134.1	C	
4'	1.98, <i>m</i> , 2H	39.6	39.6	CH ₂	C-10', C-5', C-2', C-3'
5'	2.07, <i>m</i> , 2H	26.4	26.4	CH ₂	C-4', C-6', C-7', C-3'
6'	5.06, <i>t</i> , <i>J</i> =6.9 Hz, 1H	124.3	124.3	CH	
7'		130.7	130.6	C	
8'	1.57, <i>s</i> , 3H	24.9	24.9	CH ₃	C-9', C-6', C-7'
9'	1.54, <i>s</i> , 3H	16.8	16.9	CH ₃	C-8', C-6', C-7'
10'	1.85, <i>s</i> , 3H	15.7	15.8	CH ₃	C-4', C-2', C-3'
1''	4.20, <i>t</i> , <i>J</i> =6.4 Hz, 2H	70.4		CH ₂	C-3'', C-2'', C-6
2''	1.93, <i>m</i> , 2H	22.2		CH ₂	C-3'', C-1''
3''	1.13, <i>t</i> , <i>J</i> =7.4 Hz, 3H	9.9		CH ₃	C-1'', C-2''

¹³C NMR* rubraxanthone (**2**)

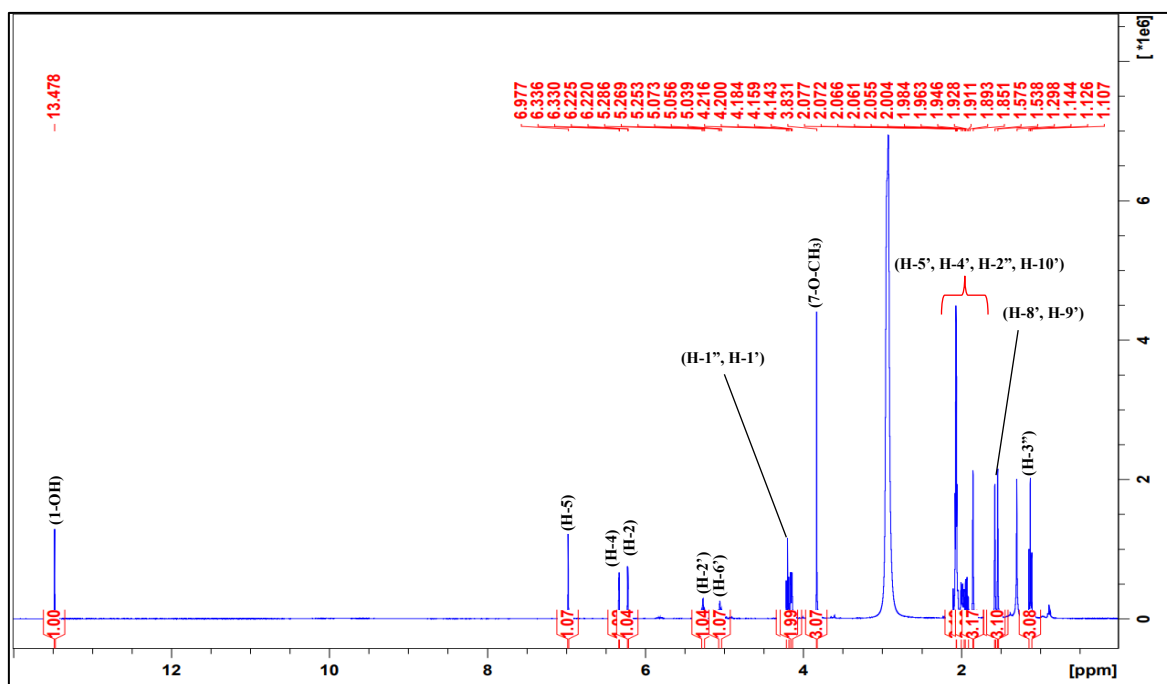


Figure 4.44: ^1H NMR of compound **81**

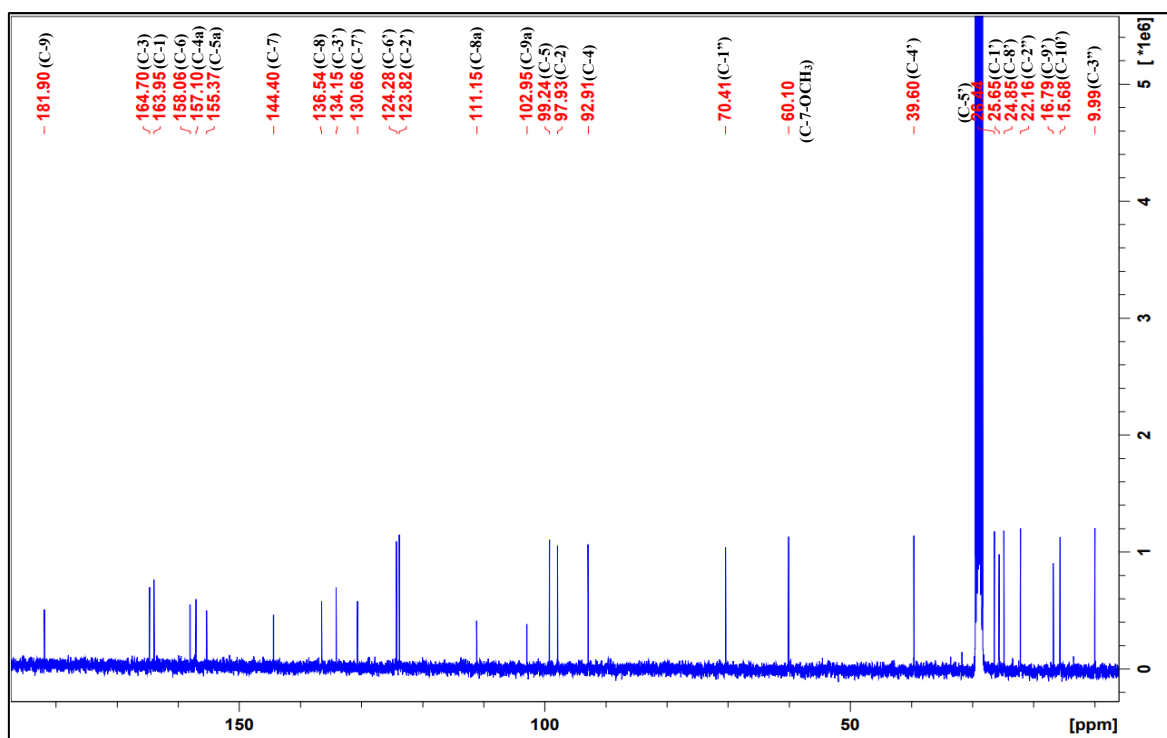


Figure 4.45: ^{13}C NMR of compound **81**

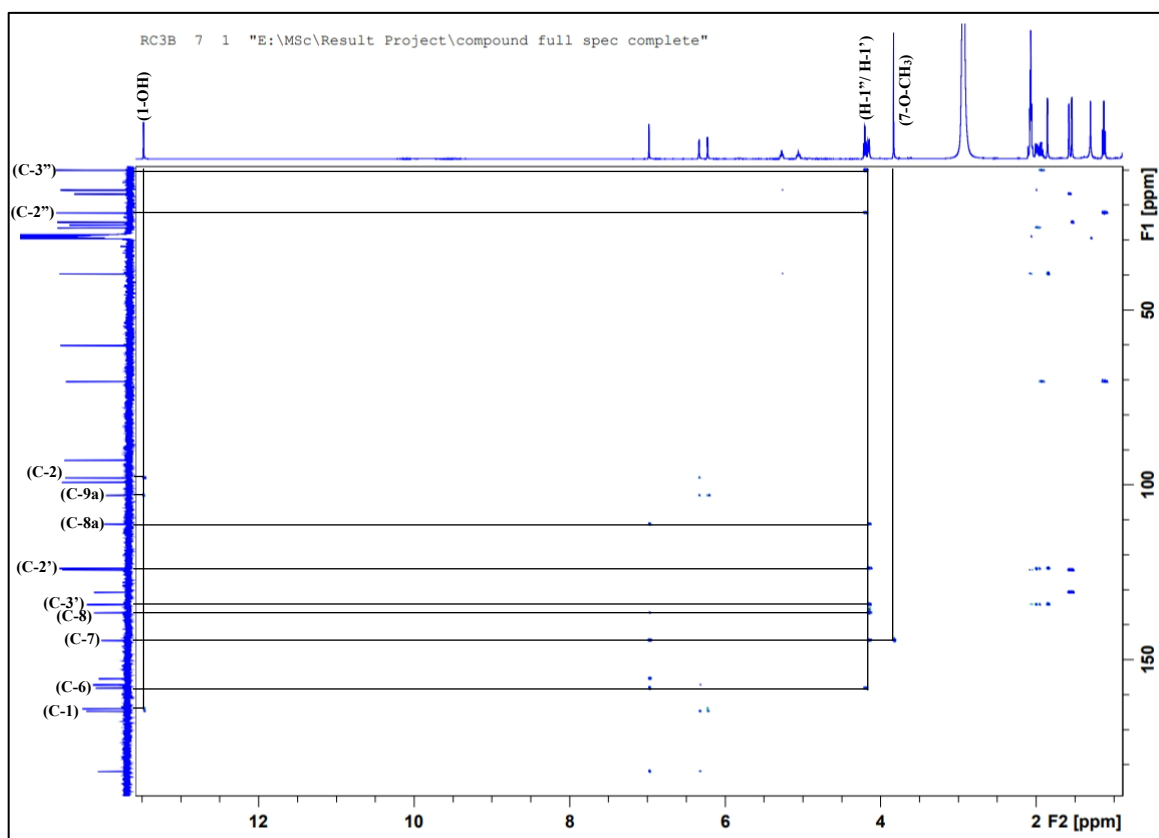


Figure 4.46: HMBC spectrum of compound **81**

4.3.2 Characterization of 3,6-*O*-pentyl-rubraxanthone (**82**)

3,6-*O*-pentyl-rubraxanthone (**82**) resulted from the rubraxanthone (**2**) reaction with 1-bromopentane. Given the structure of **82**, the synthesis appears to have selectively alkylated both C-3 and C-6 position, suggesting it's the major product of the reaction (Zhao et al., 2009). Compound **82** (percentage yield = 13.81 %) was obtained as orange wax, with a melting point of 119 °C. The LC-MS spectrum shows a molecular peak at m/z 550.35, in accordance with the molecular formula of $C_{34}H_{46}O_6$ (Figure 4.47).

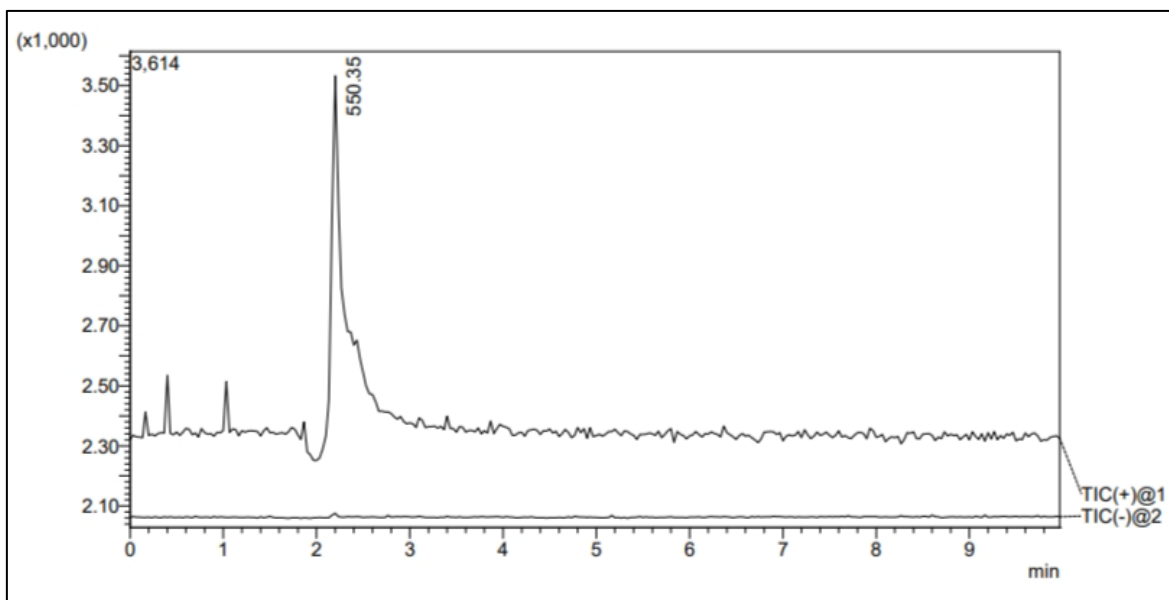


Figure 4.47: LC-MS of compound **82**

The FT-IR (Figure 4.48) shows absorption bands at 2927.57 cm^{-1} , 1646.03 cm^{-1} , 1459.04 cm^{-1} , and 1040.81 cm^{-1} which indicate the presence of C-H stretch, carbonyl group, aromatic C=C and C-O stretch, respectively. The UV spectrum gave the λ_{max} at 245 and 310 nm, suggesting the characteristics of xanthone.

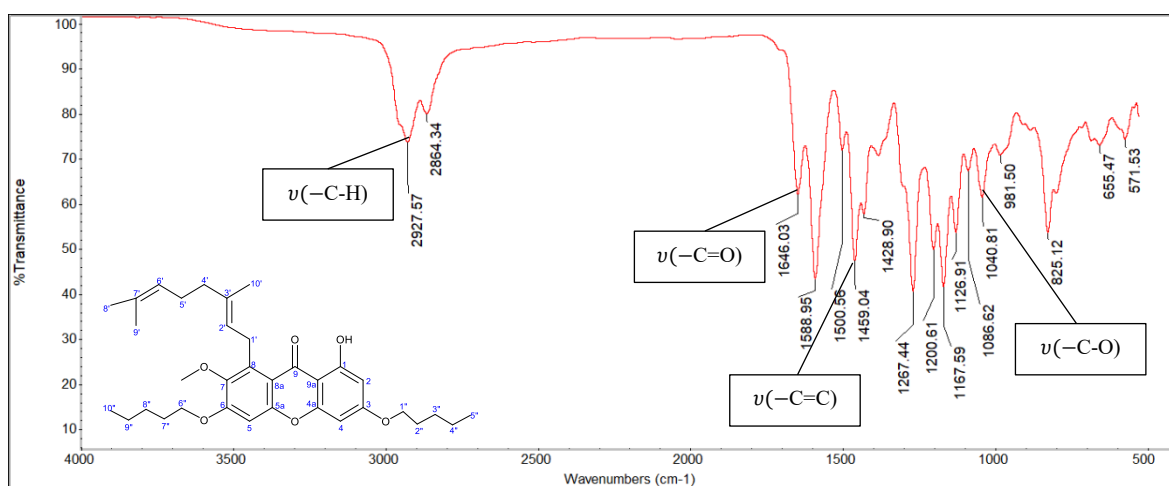


Figure 4.48: FT-IR of compound 82

In ^1H NMR, a triplet at δ 4.15 (H-6'') and a multiplet at δ 4.07 (H-1'') that differ from the rubraxanthone (**2**) spectrum shows the presence of two pentane side chain in the structure. Moreover, in ^{13}C NMR spectra, additional peaks were observed at δ 68.4 (C-1'') and δ 68.8 (C-6''), which correspond to the value of C-O (Santos et al., 2022). The pentyl group substituted the hydroxyl hydrogen previously at C-3 and C-6. This was proven from the HMBC analysis (Figure 4.52), where δ 4.07 (H-1'') has ^3J correlations with δ 165.4 (C-3) deduced that the pentyl side chain was connected to C-3. Meanwhile, δ 4.15 (H-6'') has ^3J correlations with δ 157.9 (C-6) another pentyl side chain was connected to C-6. The HMBC correlation of compound **82** is shown in Figure 4.49.

A comparison of the ^1H (Figure 4.50) and ^{13}C (Figure 4.51) NMR spectra of compound **82** and compound **2** concluded that rubraxanthone (**2**) was successfully converted into 3,6-*O*-pentyl-rubraxanthone (**82**). The data are summarized in Table 4.9.

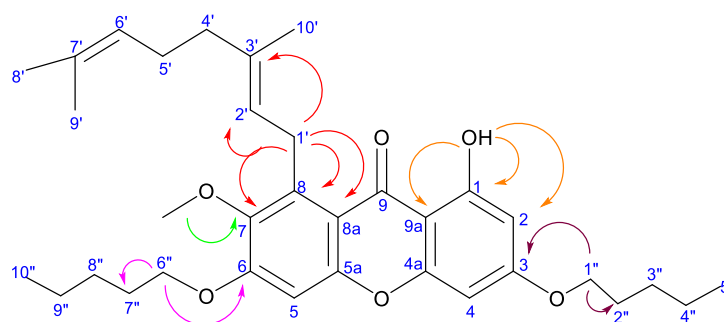


Figure 4.49: HMBC correlation in compound **82**

Table 4.9: 1D and 2D-NMR assignments of 3,6-*O*-pentyl-rubraxanthone (**82**)

Position	¹ H NMR	¹³ C NMR	¹³ C NMR*	DEPT	HMBC
1-OH	13.39, <i>s</i> , 1H	163.6	163.9	C	C-2, C-9a, C-1
2	6.19, <i>d</i> , <i>J</i> =2.1 Hz, 1H	96.9	97.8	CH	C-4, C-9a, C-1, C-3
3		165.4	164.3	C	
4	6.27, <i>d</i> , <i>J</i> =2.0 Hz, 1H	91.8	92.9	CH	C-2, C-9a, C-4a, C-3
4a		156.7	156.9	C	
5	6.80, <i>s</i> , 1H	99.0	101.9	CH	C-8a, C-7, C-5a, C-6
5a		155.2	155.3	C	
6		157.9	156.4	C	
7		144.3	143.6	C	
7-O-CH ₃	3.81, <i>s</i> , 3H	60.1	60.5	CH ₃	C-7
8		136.4	137.2	C	
8a		111.1	111.1	C	
9		181.8	181.7	C	
9a		103.4	102.9	C	
1'	4.10, <i>m</i> , 2H	25.7	25.9	CH ₂	C-8a, C-2', C-3', C-8, C-7
2'	5.28, <i>t</i> , <i>J</i> =6.2, 6.6 Hz, 1H	123.9	124.0	CH	C-10', C-4', C-8
3'		134.1	134.1	C	
4'	1.99, <i>m</i> , 2H	39.7	39.6	CH ₂	C-10', C-5', C-2', C-6', C-3'
5'	2.07, <i>m</i> , 2H	26.5	26.4	CH ₂	C-4', C-6', C-7', C-3'
6'	5.05, <i>t</i> , <i>J</i> =6.9, 7.0 Hz, 1H	124.3	124.3	CH	
7'		130.7	130.6	C	
8'	1.58, <i>s</i> , 3H	24.9	24.9	CH ₃	C-9'
9'	1.54, <i>s</i> , 3H	16.8	16.9	CH ₃	C-8'
10'	1.85, <i>s</i> , 3H	15.7	15.8	CH ₃	C-4', C-2', C-3'
1''	4.07, <i>m</i> , 2H	68.4		CH ₂	C-2'', C-3
2''/ 7''	1.55, <i>m</i> , 4H	28.1		CH ₂	C-9''
3''	1.81, <i>m</i> , 2H	28.6		CH ₂	C-1'', C-4''
4''	1.46, <i>m</i> , 2H	27.9		CH ₂	C-5'', C-2'', C-1''
5''/ 10''	0.97, <i>m</i> , 6H	13.4		CH ₃	C-2'', C-4'', C-7''
6''	4.15, <i>t</i> , <i>J</i> =6.4 Hz, 2H	68.8		CH ₂	C-7'', C-6
8''	1.91, <i>m</i> , 2H	28.6		CH ₂	C-9'', C-7'', C-1''
9''	1.45, <i>m</i> , 2H	22.2		CH ₂	C-10'', C-7''

¹³C NMR* rubraxanthone (**2**)

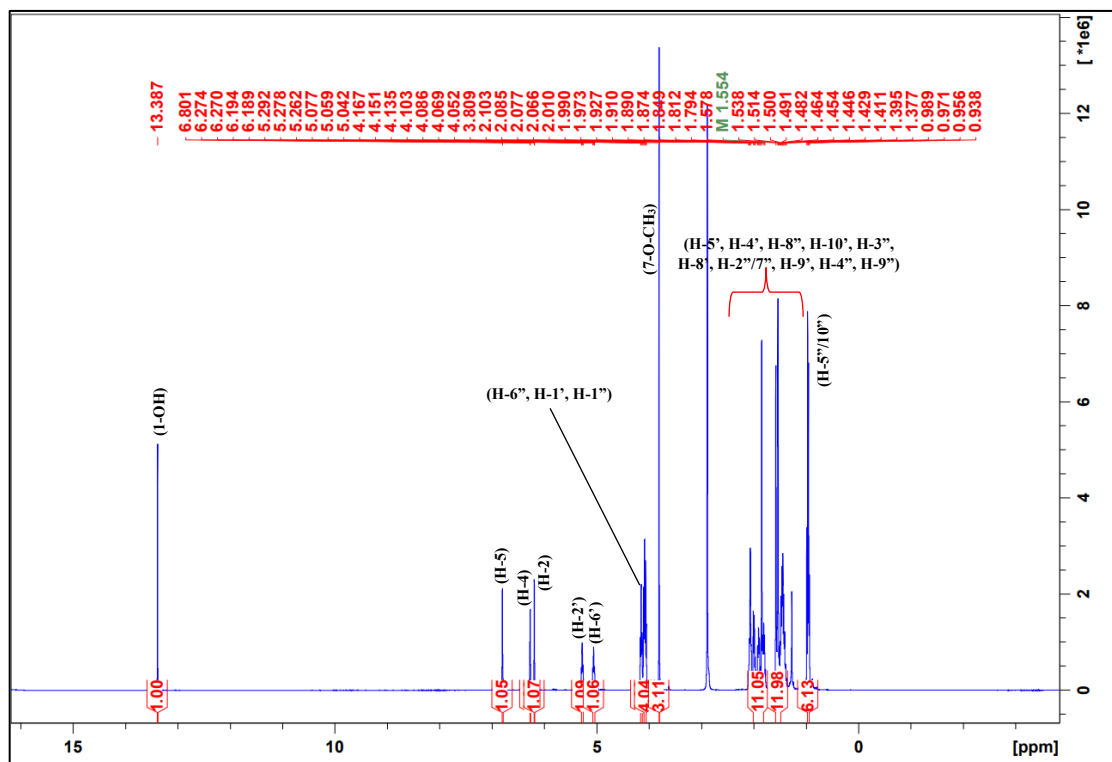


Figure 4.50: ¹H NMR of compound 82

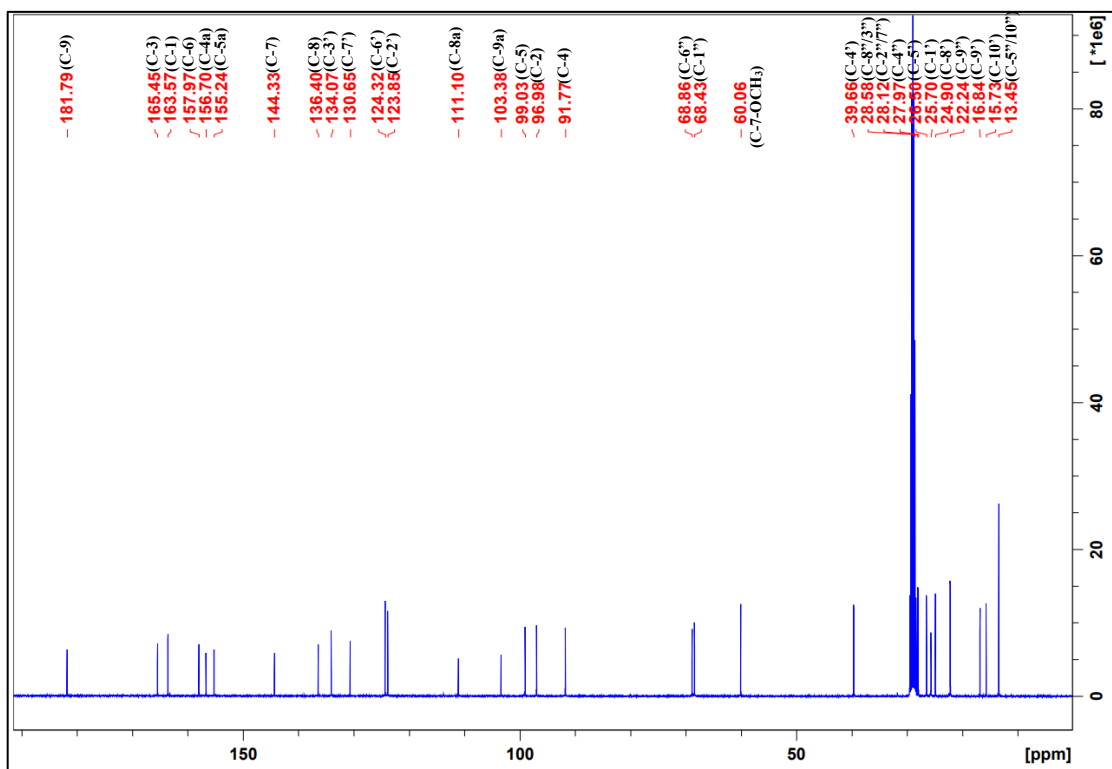


Figure 4.51: ¹³C NMR of compound 82

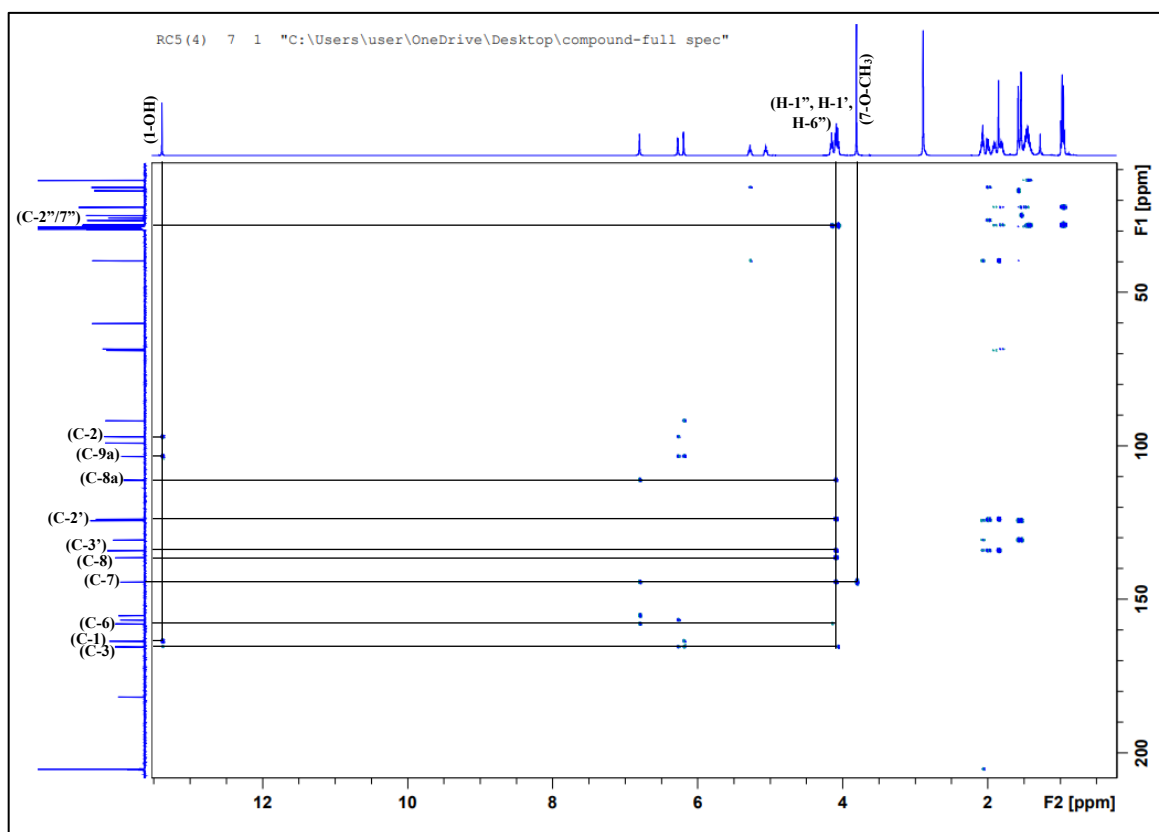


Figure 4.52: HMBC spectrum of compound **82**

4.3.3 Characterization of 3,6-*O*-hexyl-rubraxanthone (**83**)

3,6-*O*-hexyl-rubraxanthone (**83**) (115.1 mg, 39.8 %) was obtained as yellow wax from the reaction of rubraxanthone (**2**) with 1-bromohexane. It has a melting point of 214 °C. The molecular formula was determined to be C₃₆H₅₀O₆ as supported by the LC-MS spectrum which showed a molecular peak at *m/z* 578.35 (Figure 4.53).

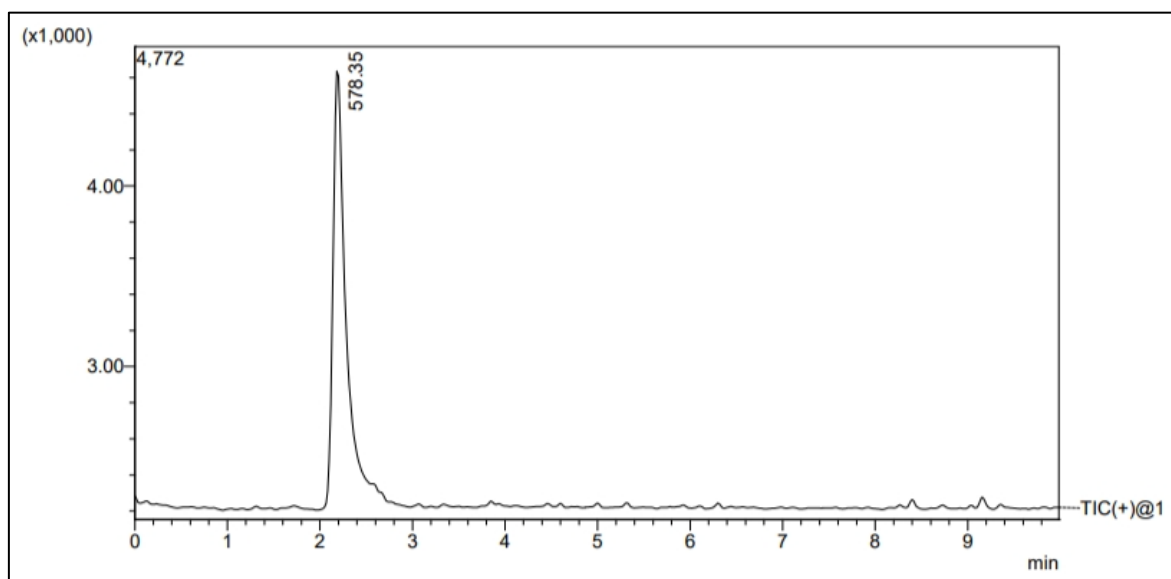


Figure 4.53: LC-MS of compound **83**

The functional group of **83** was determined from the FT-IR spectrum (Figure 4.54). They are 2923.01 cm^{-1} , 1651.99 cm^{-1} , 1460.11 cm^{-1} , 1040.15 cm^{-1} which indicate the presence of C-H stretch, C=O stretch, C=C stretch and C-O stretch, respectively. The UV spectrum, on the other hand, gave the λ_{max} at 240 and 310 nm.

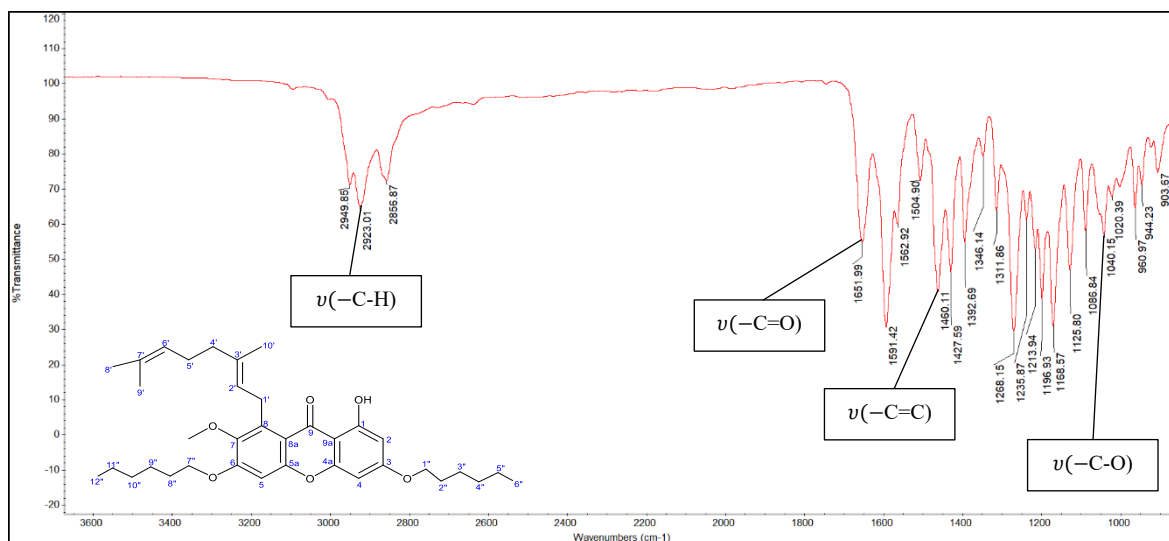


Figure 4.54: FT-IR of compound **83**

A pair of doublets at δ 4.22 (H-1'') and δ 4.12 (H-7'') in the ^1H NMR referred to the presence of two hexane side chains in the structure. In addition, additional peaks at δ 68.9 (C-1'') and δ 68.5 (C-7'') consistent with the chemical shifts of carbon atoms bonded to oxygen (C-O) were found in the ^{13}C NMR. The spectral data ^1H and ^{13}C NMR were presented in Figure 4.56 and Figure 4.57. The positioning of the hexyl side chain at position C-3 and C-6 were confirmed by HMBC analysis (Figure 4.58) and the correlation can be seen in Figure 4.55. The observation of a three-bond (^3J) correlation between δ 4.22 (H-1'') and δ 158.1 (C-6) established the connection of one hexyl group to C-6. Similarly, the presence of a ^3J correlation between δ 4.12 (H-7'') and δ 165.6 (C-3) confirmed the attachment of the other pentyl group to C-3.

The combined analysis of ^1H NMR, ^{13}C NMR, and HMBC spectra provided conclusive evidence for the presence of two hexane side chains attached to C-3 and C-6 of compound **83**, replacing the previously present hydroxyl groups in rubraxanthone (**2**). All the NMR data for compound **83** are listed in Table 4.10.

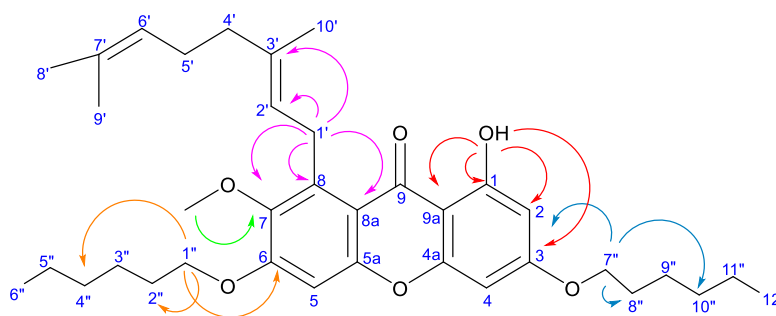


Figure 4.55: HMBC correlation in compound **83**

Table 4.10 1D and 2D-NMR assignments of 3,6-*O*-hexyl-rubraxanthone (**83**)

Position	¹ H NMR	¹³ C NMR	¹³ C NMR*	DEPT	HMBC
1-OH	13.43, <i>s</i> , 1H	163.7	163.9	C	C-2, C-9a, C-1, C-3
2	6.26, <i>d</i> , <i>J</i> =2.3 Hz, 1H	97.0	97.8	CH	C-9a, C-1, C-3
3		165.6	164.3	C	
4	6.37, <i>d</i> , <i>J</i> =2.2 Hz, 1H	91.9	92.9	CH	C-2, C-9a, C-4a, C-3
4a		156.9	156.9	C	
5	6.93, <i>s</i> , 1H	99.2	101.9	CH	C-8a, C-7, C-8, C-5a, C-6, C-9
5a		155.4	155.3	C	
6		158.1	156.4	C	
7		144.4	143.6	C	
7-O-CH ₃	3.83, <i>s</i> , 3H	60.1	60.5	CH ₃	C-7
8		136.5	137.2	C	
8a		111.2	111.1	C	
9		181.9	181.7	C	
9a		103.4	102.9	C	
1'	4.14, <i>m</i> , 2H	25.7	25.9	CH ₂	C-8a, C-2', C-3', C-8, C-7
2'	5.28, <i>t</i> , <i>J</i> =6.1, 6.6 Hz, 1H	123.7	124.0	CH	C-10', C-4'
3'		134.2	134.1	C	
4'	1.99, <i>m</i> , 2H	39.6	39.6	CH ₂	C-10', C-5', C-2', C-3'
5'	2.07, <i>m</i> , 2H	26.5	26.4	CH ₂	C-9', C-4', C-6', C-7', C-3'
6'	5.06, <i>t</i> , <i>J</i> =6.9 Hz, 1H	124.3	124.3	CH	
7'		130.7	130.6	C	
8'	1.58, <i>s</i> , 3H	24.9	24.9	CH ₃	C-9', C-3''/9'', C-5''/11'', C-4', C-6', C-7'
9'	1.54, <i>s</i> , 3H	16.8	16.9	CH ₃	C-8', C-5''/11'', C-6', C-7'
10'	1.85, <i>s</i> , 3H	15.7	15.8	CH ₃	C-1', C-5''/11'', C-7'', C-4', C-2', C-3'
1''	4.22, <i>t</i> , <i>J</i> =6.4 Hz, 2H	68.9		CH ₂	C-4'', C-2'', C-6
2''	1.90, <i>m</i> , 2H	28.8		CH ₂	C-1', C-5''/11'', C-1''
3''/9''	1.39, <i>m</i> , 4H	22.4		CH ₂	C-5''/11''
4''	1.60, <i>m</i> , 2H	25.6		CH ₂	C-2'', C-5''/11'', C-1''
5''/11''	1.40, <i>m</i> , 6H	31.4		CH ₂	C-5''/11'', C-6''/12''
6''/12''	0.94, <i>m</i> , 6H	13.4		CH ₃	C-3''/9'', C-5''/11''
7''	4.12, <i>m</i> , 2H	68.5		CH ₂	C-10'', C-8'', C-3
8''	1.80, <i>m</i> , 2H	28.8		CH ₂	C-10'', C-5'', C-7''
10''	1.51, <i>m</i> , 2H	25.5		CH ₂	C-3''/9'', C-5''/11''

¹³C NMR* rubraxanthone (**2**)

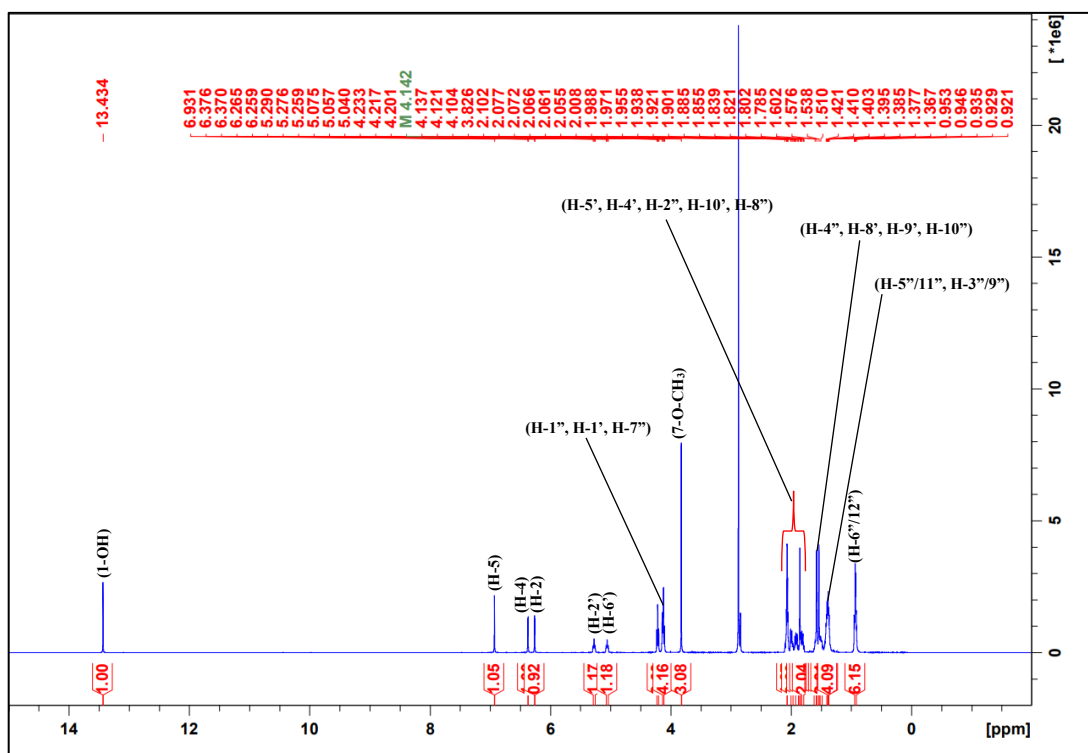


Figure 4.56: ^1H NMR of compound 83

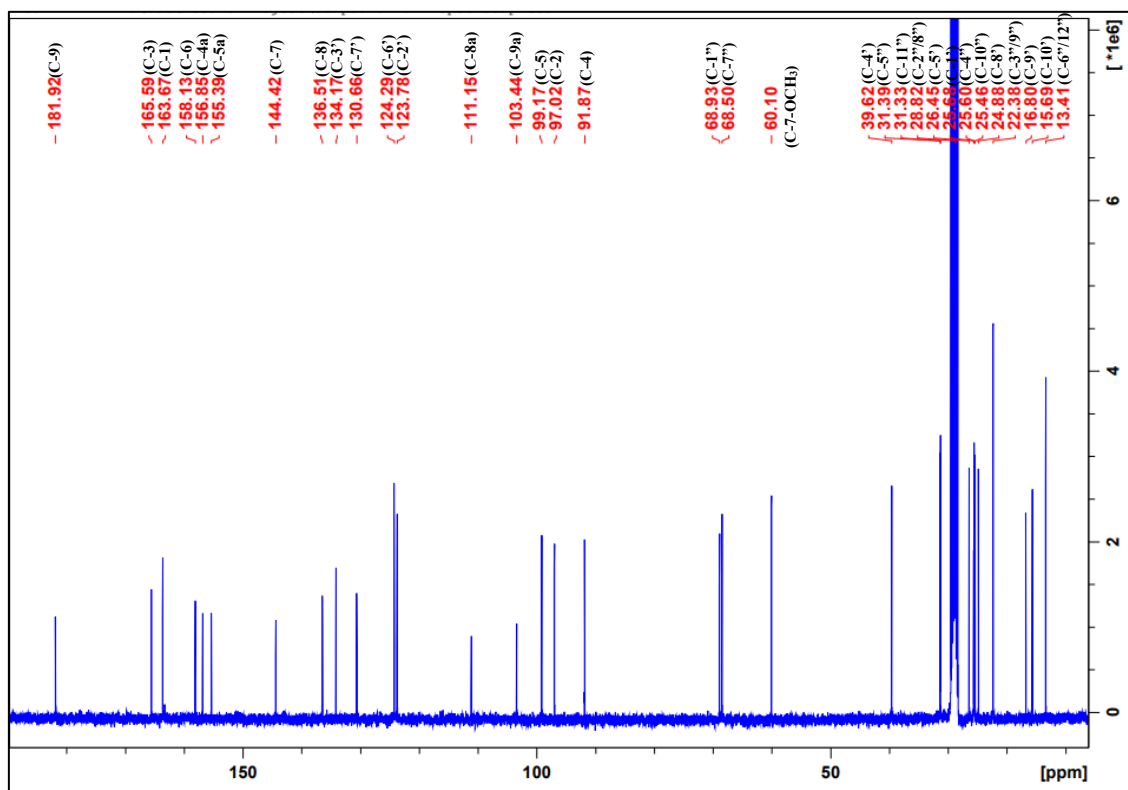


Figure 4.57: ^{13}C NMR of compound 83



4.3.4 Characterization of 6-*O*-butyl-rubraxanthone (**84**)

Structural modification of compound **2** with 1-chlorobutane and triethylamine (TEA) yielded a light-yellow powder compound, 6-*O*-butyl-rubraxanthone (**84**), with a melting point of 214 °C. 0.6 mg with a percentage yield of 0.51 % was successfully synthesized. TEA was used in the reaction due to its ability to solvate the leaving group during nucleophilic substitution reactions by deprotonation thereby stabilizing it and facilitating its detachment from the alkyl halide (Bentley et al., 2005). The LC-MS spectrum (Figure 4.59) showed a molecular peak at m/z 466.25, suggesting the molecular formula of $C_{28}H_{34}O_6$.

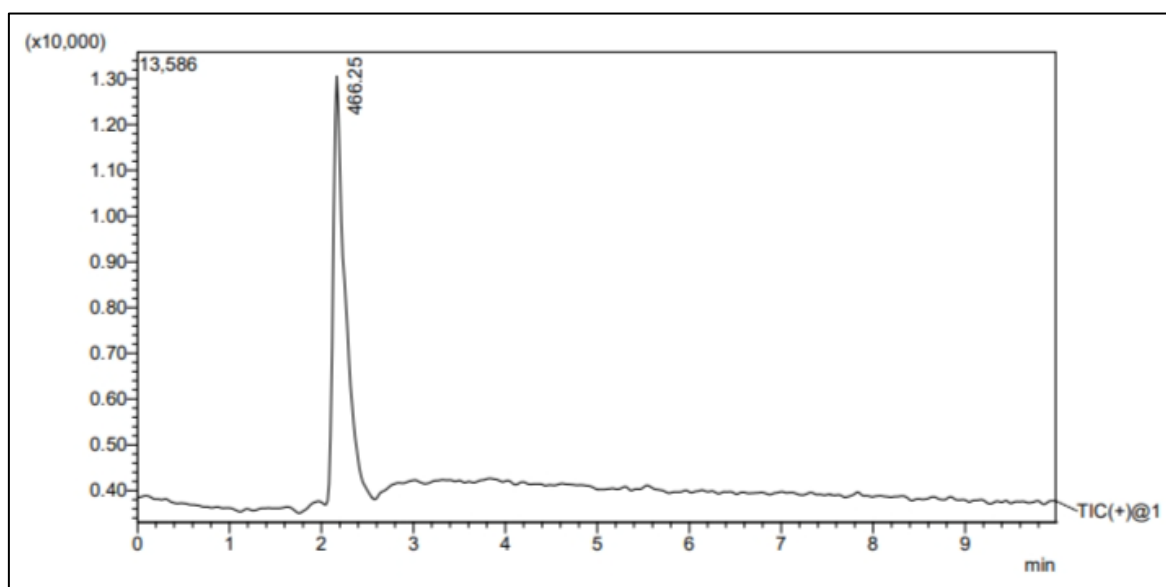


Figure 4.59: LC-MS of compound **84**

The FT-IR spectrum in Figure 4.60 showed similar signals to those for rubraxanthone **2** and other semi-synthesis compound (**80-83**). The FT-IR exhibited bands for OH stretch (3382.95 cm^{-1}), C-H stretch (2928.54 cm^{-1}), C=O stretch (1641.85 cm^{-1}), C=C stretch (1458.69 cm^{-1}) and C-O stretch (1084.41 cm^{-1}). As for the UV spectrum, it gave the λ_{max} at 237 and 310 nm, indicating the presence of xanthone's chromophore in it.

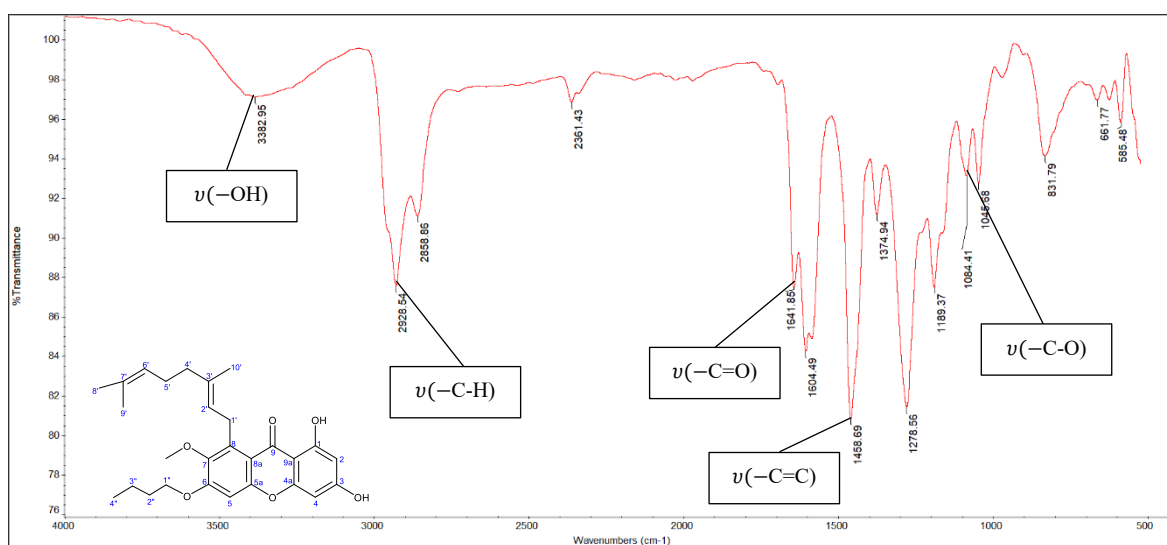
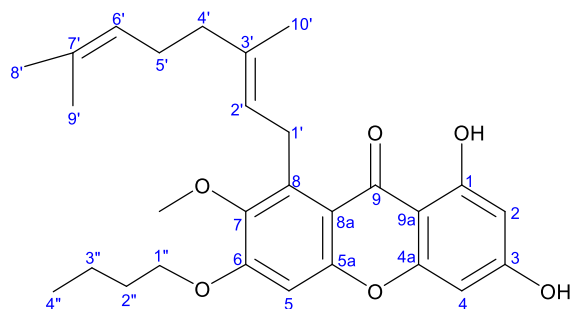


Figure 4.60: FT-IR of compound **84**

The ^1H NMR (Figure 4.61 and Table 4.11) of compound **84** showed an additional triplet at δ 4.21 (H-1'') and δ 0.99 (H-4'') indicating the additional butyl chain in the compound. However, there is no ^{13}C NMR for compound **84** due to the insufficient yield. The position of the substituent at C-6 was by comparison with rubraxanthone (**2**) and all its derivatives **81**, **82**, and **83**. The H-1'' attached to C-6 was observed at δ 4.21, which was similar to other derivatives that show the proton value of δ 4.20, δ 4.15 and δ 4.22 (compound **81**, **82**, and **83**).

Table 4.11: ^1H NMR assignments of 6-*O*-butyl-rubraxanthone (**84**)



Position	^1H NMR	^1H NMR*
1-OH		13.47, s, 1H
2	6.18, <i>d</i> , $J=2.3$ Hz, 1H	6.15, <i>d</i> , $J=2.2$ Hz, 1H
3		
4	6.29, <i>d</i> , $J=2.3$ Hz, 1H	6.23, <i>d</i> , $J=2.2$ Hz, 1H
4a		
5	6.96, <i>s</i> , 1H	6.73, <i>s</i> , 1H
5a		
6		
7		
7-O-CH ₃	3.78, <i>s</i> , 3H	3.79, <i>s</i> , 3H
8		
8a		
9		
9a		
1'	4.11, <i>d</i> , $J=6.3$ Hz, 2H	4.07, <i>d</i> , $J=6.4$ Hz, 2H
2'	5.23, <i>t</i> , $J=6.2$ Hz, 1H	5.27, <i>t</i> , $J=5.6, 6.4$ Hz, 1H
3'		
4'	1.94, <i>m</i> , 2H	1.97, <i>m</i> , 2H
5'	1.96, <i>m</i> , 2H	2.02, <i>m</i> , 2H
6'	5.01, <i>t</i> , $J=6.9$ Hz, 1H	5.00, <i>t</i> , $J=6.6, 6.8$ Hz, 1H
7'		
8'	1.53, <i>s</i> , 3H	1.53, <i>s</i> , 3H
9'	1.50, <i>s</i> , 3H	1.49, <i>s</i> , 3H
10'	1.85, <i>m</i> , 3H	1.80, <i>s</i> , 3H
1''	4.21, <i>t</i> , $J=6.3$ Hz, 2H	
2''	1.89, <i>m</i> , 2H	
3''	1.88, <i>m</i> , 2H	
4''	0.99, <i>t</i> , $J=7.2$ Hz, 3H	

^1H NMR* rubraxanthone (**2**)

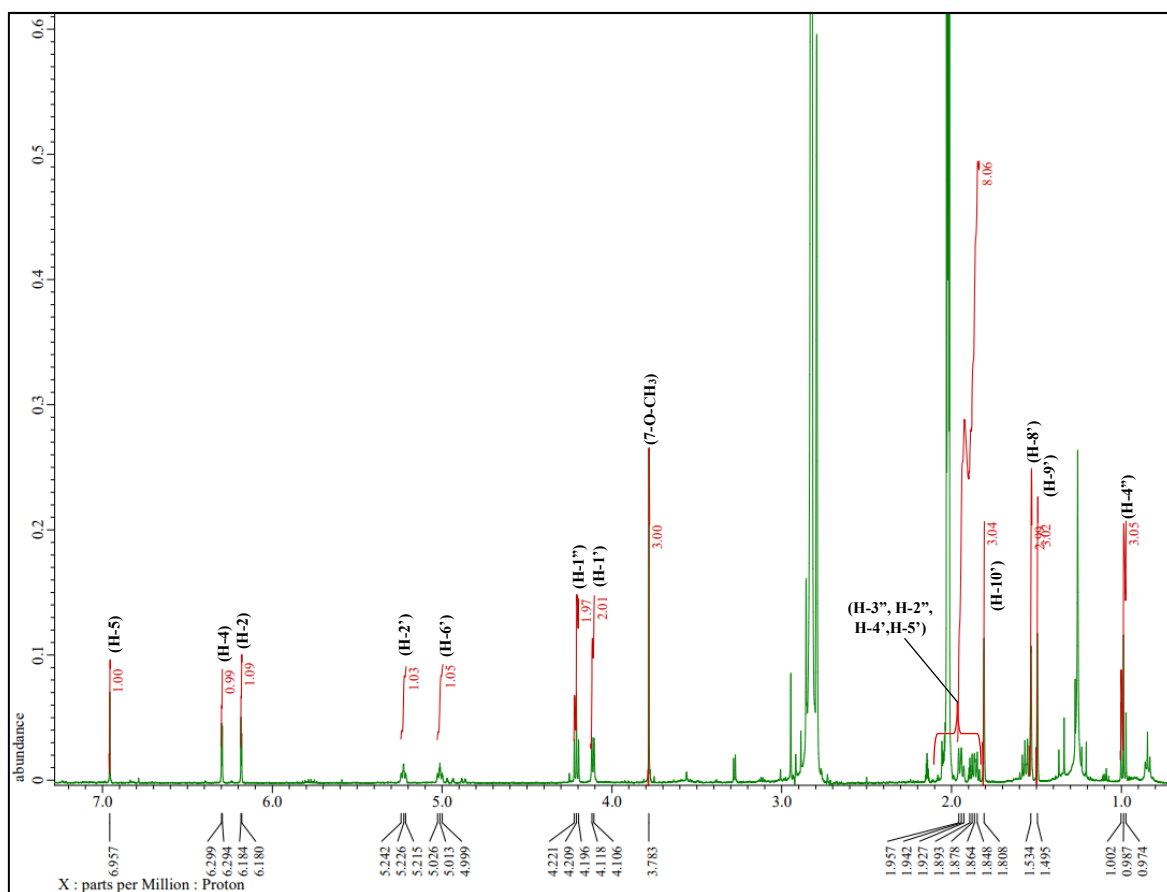


Figure 4.61: ^1H NMR spectrum of compound **84**

4.4 Anti-bacterial Assay

Anti-bacterial activity of the crude extract and isolated compounds (**76**, **2**, **77**, **78**, **79**, and **59**), as well as the semi-synthesized (**80-83**) compounds of *G. dryobalanoides*, were studied against Gram-positive and Gram-negative bacteria, which include *Lactiplantibacillus plantarum*, *Enterobacter cloacae*, *Serratia marcescens*, and *Pseudomonas aeruginosa* using diffusion and dilution method. In this study, ampicillin was used as a positive control because it has a non-selectivity effect and is effective against both Gram-positive and Gram-negative bacteria strains (Gurunathan et al., 2014). Two distinct positive control concentrations were employed (1000 µg/mL and 100 µg/mL). This differentiation was attributed to the observation that the crude extract exhibits bioactivity at concentrations below 1000 µg/mL, whereas the isolated compound demonstrates bioactivity at concentrations below 100 µg/mL (Porrás et al., 2021). DMSO, on the other hand, was used as the negative control due to it has minimal biological activity where it doesn't show any activity during biological testing (Verheijen et al., 2019).

4.4.1 Agar Well Diffusion Method

The agar well diffusion method was employed to evaluate the anti-microbial activity of the crude extracts, isolated compounds, and semi-synthesized compounds. The anti-microbial activity was evaluated by measuring the diameter of the inhibition zone after incubating the plate at 37 °C for 24 hours (Appendix E). The crude extract was screened at 1000 µg/mL, while the isolated and semi-synthesized compounds were at 100 µg/mL. The anti-microbial testing was performed in triplicate, and the results are presented as mean ± standard deviation in Table 4.12 (for plant extracts) and Table 4.13 (for isolated and semi-synthesized compounds).

Table 4.12 reveals anti-bacterial activity by all extracts against Gram-positive and Gram-negative bacterial strains, indicating strong and moderate activity (Zamakshshari et al., 2022). This broad-spectrum efficacy could be attributed to a synergistic effect among the various bioactive compounds present, as reported by Gorlenko et al. (2020). Usually, the plant extract is prone to be more effective against Gram-positive strains rather than Gram-negative strains. This is because lipopolysaccharides in hydrophilic cell walls of Gram-negative bacteria limit the passage of hydrophobic oils and prevent plant extract build-up in target cell membranes (Nurakmal et al., 2022). Interestingly, in this study, all the extract demonstrates inhibition activities towards all Gram-negative bacteria, especially *P. aeruginosa*. This unexpected finding suggests potential differences in the bacteria's cell wall structure, such as peptidoglycan composition, the presence of an outer membrane, or variations in receptors, lipids, cross-linking, or autolytic enzyme activity. As discussed by Nguyen et al. (2021), these factors could significantly influence the penetration, binding, and overall action of the *G. dryobalanoides* extracts.

Table 4.12: Anti-microbial activity of plant extract

Sample	Diameter of zone inhibition (mm) of tested bacteria			
	Gram-positive bacteria	Gram-negative bacteria		
	<i>L. plantarum</i>	<i>E. cloacae</i>	<i>P. aeruginosa</i>	<i>S. marcescens</i>
GDH	8.33 ± 0.58	8.33 ± 0.58	10.33 ± 0.58	8.00 ± 0.00
GDEA	8.67 ± 0.58	9.00 ± 0.00	12.67 ± 0.58	9.00 ± 1.00
GDM	8.67 ± 0.58	8.33 ± 0.58	13.33 ± 1.53	8.33 ± 0.58
DMSO	NA	NA	NA	NA
Ampicillin	25.00 ± 0.00	27.33 ± 1.15	46.33 ± 1.15	26.33 ± 0.58
Notes: GDH: <i>G. dryobalanoides</i> hexane extract; GDEA: <i>G. dryobalanoides</i> ethyl acetate extract; GDM: <i>G. dryobalanoides</i> methanol extract; DMSO: Dimethyl sulfoxide; Ampicillin: concentration 1000 µg/mL ; NA: No activity				

Similarly, the isolated compounds from *G. dryobalanoides* show bacterial inhibition against all tested bacterial strains (Table 4.13). Among all the isolated compounds, rubraxanthone (**2**) showed significant inhibition zone towards *P. aeruginosa* with an IZD of 18.33 ± 1.53 mm. Meanwhile, 9-hydroxycalabaxanthone (**79**) displayed inhibition against *E. cloacae* with an IZD of 10.00 ± 1.00 mm. Eventually, stigmasterol (**59**) with the IZD ≥ 10.00 mm against *L. plantarum*, *E. cloacae*, and *P. aeruginosa*. It is not surprising given that rubraxanthone (**2**) and stigmasterol (**59**) have been shown to have anti-bacterial activity (Muhammad et al., 2019). This is due to the presence of hydroxyl (-OH) groups in the chemical structure of compounds **2** and **59** facilitates hydrogen bonding, which allows compounds to diffuse through bacterial membranes, resulting in cytoplasmic acidification and, eventually, cell death (Alibi et al., 2021). However, these compounds **2** and **59** have never been tested on *L. plantarum*, *E. cloacae*, and *S. marcescens*.

Interestingly, the semi-synthesized compound shows better anti-bacterial activity (moderate and strong) towards several bacterial strains. This was influenced by the alkyl chain bore to the series which contribute to the lipophilicity, where it enhances bacterial cell membrane adhesion and ultimately causes disruption (Abd Halim et al., 2023). Some derivatives surpassed the parent compound (compound **2**) against specific bacteria. For example, compound **83** displayed a comparable inhibition zone against *L. plantarum*. Compounds **80** and **81** demonstrated superior activity against *E. cloacae* compared to **2**, while compounds **82** and **83** maintained moderate activity within a range of ± 0.33 difference. Conversely, all derivatives showed decreased activity against *P. aeruginosa* compared to **2**, although **82** and **83** demonstrated comparable levels inhibitory effect with compound **2**. In contrast, all derivatives showed significantly higher inhibition against *S. marcescens* than **2**. The susceptibility of bacteria can vary significantly due to diverse

resistance mechanisms. The presence of specific resistance genes or efflux pumps can influence a bacterium's overall sensitivity, resulting in variable inhibition zone diameters of the bacteria (Endo et al., 2024).

The encouraging agar well diffusion assay results revealed a broad-spectrum activity of most *G. dryobalanoides* extracts, isolated and synthesized compounds against the tested bacterial strains. These extracts and phytochemicals effectively inhibited the growth of nearly all microorganisms, with inhibition zone diameters ranging from 7 to 18 mm. The moderate and strong IZD results were further investigated for their Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays. The MIC was used to determine the bacteriostatic effect, while the MBC was used to determine the bactericidal effect of extract and compounds. The compounds and extracts were considered active if inhibition zone diameters were 10 mm or greater (Zamakshshari et al., 2022).

Table 4.13 Anti-microbial activity of isolated and synthesized compound

Sample	Diameter of zone inhibition (mm) of tested bacteria			
	Gram-positive bacteria	Gram-negative bacteria		
	<i>L. plantarum</i>	<i>E. cloacae</i>	<i>P. aeruginosa</i>	<i>S. marcescens</i>
76	7.66 ± 0.58	8.33 ± 1.15	7.33 ± 0.58	8.00 ± 0.00
2	9.67 ± 0.58	8.33 ± 0.58	18.33 ± 1.53	7.33 ± 0.58
77	8.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	8.00 ± 0.00
78	9.67 ± 1.53	7.67 ± 0.58	7.67 ± 0.58	7.67 ± 0.58
79	8.00 ± 0.00	10.00 ± 1.00	7.00 ± 0.00	8.00 ± 0.00
59	10.33 ± 0.58	10.00 ± 0.00	15.33 ± 2.08	8.67 ± 0.58
80	8.00 ± 1.00	10.00 ± 1.00	6.67 ± 0.58	8.00 ± 0.00
81	8.33 ± 1.53	9.00 ± 1.00	7.33 ± 0.58	8.00 ± 0.00
82	7.67 ± 0.58	8.00 ± 0.00	16.33 ± 1.53	7.66 ± 0.58
83	9.66 ± 0.58	8.00 ± 0.00	15.67 ± 1.15	8.00 ± 0.00
DMSO	NA	NA	NA	NA
Ampicillin	13.00 ± 0.00	7.67 ± 0.58	37.00 ± 3.00	14.00 ± 0.00
Notes: DMSO: Dimethyl sulfoxide; Ampicillin: concentration 100 µg/mL; NA: No activity				

4.4.2 Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

All of the crude extracts underwent MIC testing against *P. aeruginosa*, as it demonstrated greater activity against this strain than others. The MIC recorded for GDEA and GDM were 31.25 and 62.5 $\mu\text{g/mL}$, respectively, against the tested bacteria corresponding to strong activity ($\text{MICs} \leq 100 \mu\text{g/mL}$). However, GDH was found to have no activity toward tested bacteria. The results of the anti-bacterial activity of the crude extract are comparable to the work (Nguyen et al., 2021; Akongwi et al., 2023), where a semi-polar crude extract (ethyl acetate) and polar crude extract (methanol) showed the most active and its activity was significantly higher than those other extracts. The differences in the anti-bacterial activity of the various extracts might be due to differences in the chemical constituents in each extract (Zamakshshari et al., 2022). In this case, this might be due to the presence of rubraxanthone (**2**) in the GDEA and GDM but not GDH which were well known for its anti-bacterial activities (Muhammad et al., 2019) that contribute to the bacteriostatic effects.

Contrariwise, among the isolated compounds, rubraxanthone (**2**), 9-hydroxycalabaxanthone (**79**), and stigmasterol (**59**) were selected for MIC and MBC determination. Conversely, compounds **80**, **82**, and **83** were chosen from the synthesized compounds for further evaluation. According to Porras et al. (2021), $\text{MICs} \leq 10 \mu\text{g/mL}$ indicate excellent anti-bacterial activity. Where, MICs between 11 and 100 $\mu\text{g/mL}$ suggest moderate activity. In this study, rubraxanthone (**2**) showed the strongest inhibitory activity against *P. aeruginosa* with an MIC value of only 6.25 $\mu\text{g/mL}$. 9-Hydroxycalabaxanthone (**79**), on the other hand, demonstrated moderate activity against *E. cloacae*, with MIC and MBC values of 25.0 $\mu\text{g/mL}$ and 100.0 $\mu\text{g/mL}$, respectively. Stigmasterol (**59**) had been

tested against both Gram-positive and Gram-negative bacteria. The results indicate that stigmasterol had moderate activities against *L. plantarum* (MIC=50.0 µg/mL; MBC=100 µg/mL), *E. cloacae* (MIC=25.0 µg/mL; MBC=100 µg/mL) and *P. aeruginosa* (MIC=25.0 µg/mL). As for the semi-synthesized compounds, **82** and **83** exhibited moderate activity against *P. aeruginosa*, with both compounds giving MIC values of 50.0 µg/mL. Meanwhile, compound **80** initially showed promising activity against *E. cloacae* in the agar well diffusion assay. However, it exhibited weak activity during MIC (MIC $\geq$ 100.0 µg/mL).

The MBC: MIC ratio is often used to evaluate a compound's bactericidal potential, with a ratio of ≤ 4 indicating bactericidal activity (Akongwi et al., 2023). This study identified compounds **59** and **79** as promising candidates against bacterial proliferation, exhibiting clear bactericidal activity. Notably, compound **59** displayed a remarkable ability to inhibit bacterial growth against both *E. cloacae* (MBC: MIC ≤ 4) and *L. plantarum* (MBC: MIC ≤ 2). This efficacy can be attributed to its structural characteristics. From the viewpoint of the SAR study, compound **59**, a phytosterol that belongs to tetracyclic triterpenes, has shown outstanding anti-microbial activity towards both groups of bacteria due to the ability to hinder bacterial cell surface protein functions and cause changes in membrane composition (Bakrim et al., 2022). Conversely, compound **79** also displayed a promising MBC: MIC ratio of ≤ 4 against *E. cloacae*, suggesting its potential as an anti-bacterial agent.

4.4.3 Structure-activity relationship (SAR) studies

The structure-activity relationships of xanthenes were studied to clarify the structural requirement for anti-bacterial activity (Figure 4.62). Saepudin et al. (2019) suggested that xanthenes can remarkably hinder bacterial cell division, effectively halting their growth. Xanthenes consist of the hydrophobic core, which is important in anti-bacterial activity as it

can disrupt the inner bacterial membrane (Koh et al., 2015). Due to strong hydrophobic interactions, the natural xanthone skeletal can disrupt lipid membranes non-selectively (Zou et al., 2013). However, their effectiveness also depends on the position of functional groups on their skeleton (Dharmaratne et al., 2013). The hydroxy group (OH) groups, substituted at C-6 and C-3, are particularly important (Muhammad et al., 2019) as they may inhibit microbial proliferation (Ruan et al., 2017). Meanwhile, anti-bacterial activity was also enhanced by the prenyl side chain at C-2 (Muhammad et al., 2019). Contra wise, xanthenes also can be modified to improve the lipophilicity of the backbone compounds and biological activities (Liu et al., 2022).

In this study, all isolated xanthenes exhibited promising anti-bacterial activity. Among all, compounds **79** and **2** proceeded to the MIC and MBC testing due to their exceptional efficacy against the tested bacteria. Compound **79** consists of an oxygen (O) group at C-6 and an unsaturated isoprenyl group at C-8, which is responsible for its potent anti-bacterial effect. Conversely, compound **2** possesses double bonds at the geranyl side chain and OH groups attachment at C-6 and C-3, which are believed to have the ability to proliferate microorganisms (Ruan et al., 2017). The anti-bacterial activity of compounds **79** and **2** is likely linked to their shared structure: a hydrophobic xanthone core tethered to lipophilic chains. The hydrophobic core is posited to disrupt the integrity of the inner bacterial membrane, while the lipophilic chains may be a critical factor facilitating the penetration across the cytoplasmic membrane (Koh et al., 2015). Besides, the presence of OH or O groups at positions C-3 and C-6 also can affect the molecule's anti-microbial efficacy.

Aforementioned earlier, structural modification of xanthenes helps to enhance the lipophilicity of their core structure, thereby potentiating their biological activities. Compound **2** had been structurally modified by adding an alkyl substituent to the structure. The rationale was to demonstrate that the presence of alkyl chains can significantly enhance anti-microbial activity, whereby, it can improve the lipophilic characteristics (Abd Halim et al., 2023). Interestingly, all the synthesized compounds exhibited strong or moderate anti-bacterial activity, as evidenced by their inhibition zones. Out of all synthesized compounds, **80**, **82**, and **83** proceeded with the MIC and MBC testing. When comparing the parent compound **2** with its derivatives (**80**, **82**, and **83**) during MIC and MBC testing, a slight decrease in activity was observed against all tested bacteria which corresponds to moderate activity. The reduction in anti-bacterial activity could be attributed to a higher molecular weight of the derivatives from bulky groups causing steric hindrance (Marianna et al., 2019) which limits the interaction with the bacteria receptor. Moreover, the absence of a free OH group at positions C-3 and C-6 could also impact the molecule's anti-microbial efficacy for the xanthone scaffold.

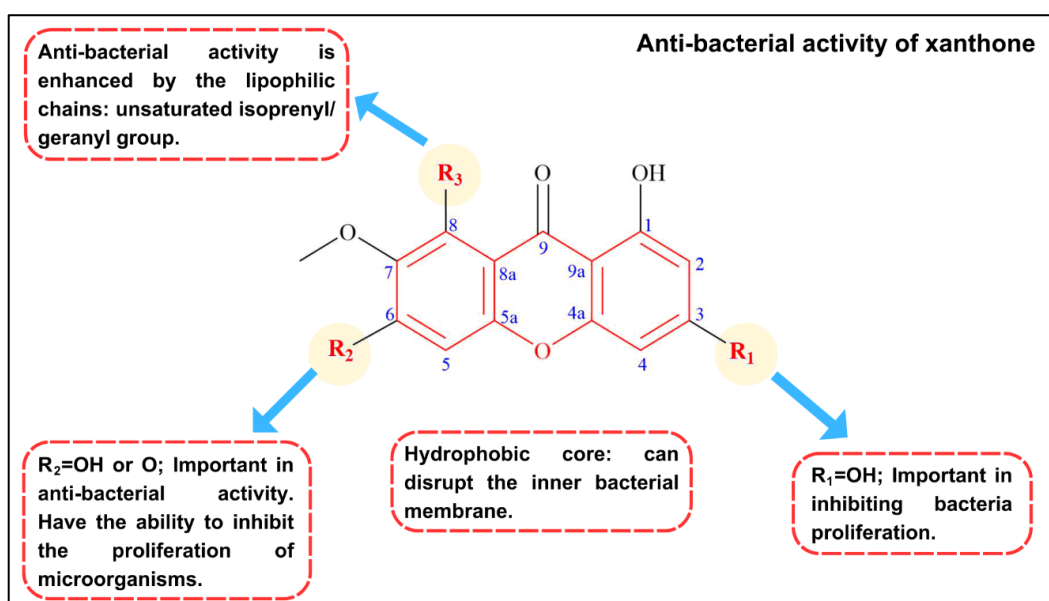


Figure 4.62: Structure-activity relationships of xanthenes

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

A detailed study on secondary metabolites was carried out on the stem bark of *Garcinia dryobalanoides*. Six compounds were successfully isolated, including one benzophenone, four xanthones, and one triterpene. They are 2-hydroxy-(4-octyloxy)benzophenone (**76**), rubraxanthone (**2**), 8-deoxygartanin (**77**), isocowanol (**78**), 9-hydroxycalabaxanthone (**79**), and stigmasterol (**59**). Astonishingly, 2-hydroxy-(4-octyloxy)benzophenone (**76**) was the first naturally occurring benzophenone isolated from plant species, especially the *Garcinia* genus. Previously, it was isolated from the *Pinus sibirica* shell.

Along with that, structural modification of a major compound isolated from *G. dryobalanoides*, rubraxanthone (**2**) was successfully synthesized *via* Williamson etherification with various alkyl halides. Five novel rubraxanthone (**2**) derivatives were obtained, namely, 3-*O*-propyl-rubraxanthone (**80**), 6-*O*-propyl-rubraxanthone (**81**), 3,6-*O*-pentyl-rubraxanthone (**82**), 3,6-*O*-hexyl-rubraxanthone (**83**) and 6-*O*-butyl-rubraxanthone (**84**) were obtained. All isolated secondary metabolites and synthesized compounds were characterized using a variety of spectroscopic analyses.

The evaluation of anti-bacterial activity on crude extracts, isolated secondary metabolites, and synthesized compounds against *Lactiplantibacillus plantarum*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Serratia marcescens* demonstrated moderate to excellent activities outlining their bacteriostatic as well as bactericidal potential.

Notably, rubraxanthone (**2**) exhibited the strongest activity during MIC testing, indicating bacteriostatic properties. On the other hand, 9-hydroxycalabaxanthone (**79**) and stigmasterol (**59**) displayed bactericidal properties with an MBC: MIC ratio equal to or less than 4 against the tested bacteria.

The study on the structure-activity relationship (SAR) found that the presence of prenyl and hydroxy groups on the xanthone led to significant bacterial inhibition. However, when an alkyl chain was introduced to the hydroxy part, it decreased the anti-bacterial activity of the compound. This decrease is likely due to the bulkiness causing steric hindrances, which limits interaction with the bacterial receptor.

5.2 Recommendations

The findings in this study suggest *G. dryobalanoides* as a potential source for developing novel anti-bacterial agents. Most of the isolated and semi-synthesized compounds have demonstrated promising anti-bacterial activities. 9-hydroxycalabaxanthone (**79**) and stigmasterol (**59**) are the most potent antimicrobial drug candidates due to its bactericidal properties. Therefore, further research is necessary to explore this compound's mechanisms of action and anti-bacterial potential. This investigation could pave the way for the development of new antibiotics aimed at combating drug-resistant pathogens.

REFERENCES

- Abd Halim, A. N., Hussin, A. S. M., Ngaini, Z., Sing, N. N., Jong, S. A., & Saberi, M. M. M. (2023). Synthesis, *in vitro* and *in silico* studies of alkylated paracetamol derivatives as potential antibacterial agent. *Borneo Journal of Resource Science and Technology*, 13(2), 161-174..
- Acuna, U. M., Jancovski, N., & Kennelly, E. J. (2009). Polyisoprenylated benzophenones from Clusiaceae: potential drugs and lead compounds. *Current Topics in Medicinal Chemistry*, 9(16), 1560-1580.
- Agidew, M. G. (2022). Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. *Bulletin of the National Research Centre*, 46(1), 1-22.
- Akongwi, M., Kwene, E. C., Awah, L. A., Tih, A. E., Ghogomu, R. T., Cho-Ngwa, F., & Ngemenya, M. N. (2023). Anti-Salmonella activity on multidrug-resistant strains and cytotoxicity of extracts and constituents of *Garcinia brevipedicellata* and *Garcinia epunctata*. *Scientific African*, 19, e01465.
- Akoro, S. M., Aiyelaagbe, O. O., Onocha, P. A., & Gloer, J. B. (2020). Gakolanone: A new benzophenone derivative from *Garcinia kola* Heckel stem-bark. *Natural product research*, 34(2), 241-250.
- Alibi, S., Crespo, D., & Navas, J. (2021). Plant-derivatives small molecules with antibacterial activity. *Antibiotics*, 10(3), 231.
- Alseekh, S., de Souza, L. P., Benina, M., & Fernie, A. R. (2020). The style and substance of plant flavonoid decoration; towards defining both structure and function. *Phytochemistry*, 174, 112347.

- Ambarwati, N. S. S., Elya, B., Malik, A., Hanafi, M., & Awang, M. S. N. (2022). Isolation, identification, and antibacterial of amentoflavone from *Garcinia latissima* Miq. leaves. *International Journal of Applied Pharmaceutics*, 14(Special Issue 3), 67-73.
- Asprion, N., Hasse, H., & Maurer, G. (2001). FT-IR spectroscopic investigations of hydrogen bonding in alcohol–hydrocarbon solutions. *Fluid Phase Equilibria*, 186(1-2), 1-25.
- Badave, P., Gaikwaid, S., & Jagtap, S. V. (2020). Versatile remarkable potent bioactivity of quinone based compounds to beat the diseases. *Engineering Managment*, 83, 25605-25608.
- Bajpai, V. K., Majumder, R., & Park, J. G. (2016). Isolation and purification of plant secondary metabolites using column-chromatographic technique. *Bangladesh Journal of Pharmacology*, 11(4), 844-848.
- Bakrim, S., Benkhaira, N., Bourais, I., Benali, T., Lee, L.-H., El Omari, N., Sheikh, R. A., Goh, K. W., Ming, L. C., & Bouyahya, A. (2022). Health benefits and pharmacological properties of stigmasterol. *Antioxidants*, 11(10), 1912.
- Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for *in vitro* evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*, 6(2), 71-79.
- Bentley, T. W., Harris, H. C., Ryu, Z. H., Lim, G. T., Sung, D. D., & Szajda, S. R. (2005). Mechanisms of solvolyses of acid chlorides and chloroformates. Chloroacetyl and phenylacetyl chloride as similarity models. *The Journal of Organic Chemistry*, 70(22), 8963-8970.
- Brejijeh, Z., Jubeh, B., & Karaman, R. (2020). Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it. *Molecules*, 25(6).

- Brito, L. C., Berenger, A. L. R., & Figueiredo, M. R. (2017). An overview of anticancer activity of *Garcinia* and *Hypericum*. *Food Chem Toxicol*, 109(Pt 2), 847-862.
- Cane, H. P. C. A., Saidi, N., Yahya, M., Darusman, D., Erlidawati, E., Safrida, S., & Musman, M. (2020). Macrophylloflavone: a new biflavonoid from *Garcinia macrophylla* Mart.(Clusiaceae) for antibacterial, antioxidant, and anti-type 2 diabetes mellitus activities. *The Scientific World Journal*, 2020(1), 2983129.
- Chaturvedula, V. S. P., & Prakash, I. (2012). Isolation of stigmasterol and β -sitosterol from the dichloromethane extract of *Rubus suavissimus*. *International Current Pharmaceutical Journal*, 1(9), 239-242.
- Conceição, M., Beserra, F. P., Aldana Mejia, J. A., Caldas, G. R., Tanimoto, M. H., Luzenti, A. M., Gaspari, P. D. M., Evans, N. D., Bastos, J. K., & Pellizzon, C. H. (2023). Guttiferones: An insight into occurrence, biosynthesis, and their broad spectrum of pharmacological activities. *Chemico-Biological Interactions*, 370, 110313.
- Cooper, R., & Nicola, G. (2014). *Natural products chemistry: sources, separations and structures*. CRC press.
- Daud, S., Karunakaran, T., Santhanam, R., Nagaratnam, S. R., Jong, V. Y. M., & Ee, G. C. L. (2021). Cytotoxicity and nitric oxide inhibitory activities of xanthenes isolated from *Calophyllum hosei* Ridl. *Natural product research*, 35(24), 6067-6072.
- Davison, E. K., & Brimble, M. A. (2019). Natural product derived privileged scaffolds in drug discovery. *Current opinion in chemical biology*, 52, 1-8.
- Delita, N., Elya, B., & Hanafi, M. (2020). Isolation and identification of chemical compounds from *garcinia fruticosa* lauterb stem bark extract. *Pharmacognosy Journal*, 12(6s), 1641-1652.

- Devi, R. S., Chakroborty, S., Kumar, S., & Dhal, N. K. (2019). *Garcinia xanthochymus* Hook. f. ex T. Anderson: an ethnobotanically important tree species of the similipal biosphere reserve, India. *Ethnopharmacology and biodiversity of medicinal plants* (pp. 385-396). Apple Academic Press.
- Dey, V., Hasnu, S., Nahar, S., & Tanti, B. (2017). Phytochemical analysis and antioxidant activities of *Garcinia morella* Desr. *International Journal Multidisciplinary Approach and Studies*, 4(4), 31-37.
- Dharmaratne, H., Sakagami, Y., Piyasena, K., & Thevanesam, V. (2013). Antibacterial activity of xanthonenes from *Garcinia mangostana* (L.) and their structure–activity relationship studies. *Natural product research*, 27(10), 938-941.
- Ding, Y., & Xue, X. (2024). Medicinal chemistry strategies for the modification of bioactive natural products. *Molecules*, 29(3), 689.
- Ecevit, K., Barros, A. A., Silva, J. M., & Reis, R. L. (2022). Preventing microbial infections with natural phenolic compounds. *Future Pharmacology*, 2(4), 460-498.
- Elmaidomy, A. H., Shady, N. H., Abdeljawad, K. M., Elzamkan, M. B., Helmy, H. H., Tarshan, E. A., Adly, A. N., Hussien, Y. H., Sayed, N. G., & Zayed, A. (2022). Antimicrobial potentials of natural products against multidrug resistance pathogens: A comprehensive review. *RSC advances*, 12(45), 29078-29102.
- Endo, S., Tada, T., Oshiro, S., Hishinuma, T., Tohya, M., Watanabe, S., Sekiguchi, J.-I., Abe, M., Nakada, K., & Kirikae, T. (2024). Evaluation of antimicrobial susceptibility tests for *Acinetobacter* and *Pseudomonas* species using disks containing a high dose of meropenem. *Scientific Reports*, 14(1), 2749.

- Fotso, G. W., Ntummy, A. N., Ngachussi, E., Dube, M., Mapitse, R., Kapche, G. D., Andrae-Marobela, K., Ngadjui, B. T., & Abegaz, B. M. (2014). Epunctanone, a new benzophenone, and further secondary metabolites from *Garcinia epunctata* Stapf (Guttiferae). *Helvetica Chimica Acta*, 97(7), 957-964.
- Gabriel, G. J., Som, A., Madkour, A. E., Eren, T., & Tew, G. N. (2007). Infectious disease: Connecting innate immunity to biocidal polymers. *Materials Science and Engineering: R: Reports*, 57(1-6), 28-64.
- Gontijo, V. S., de Souza, T. C., Rosa, I. A., Soares, M. G., da Silva, M. A., Vilegas, W., Júnior, C. V., & Dos Santos, M. H. (2012). Isolation and evaluation of the antioxidant activity of phenolic constituents of the *Garcinia brasiliensis* epicarp. *Food chemistry*, 132(3), 1230-1235.
- Gorlenko, C. L., Kiselev, H. Y., Budanova, E. V., Zamyatin Jr, A. A., & Ikryannikova, L. N. (2020). Plant secondary metabolites in the battle of drugs and drug-resistant bacteria: new heroes or worse clones of antibiotics? *Antibiotics*, 9(4), 170.
- Gurunathan, S., Han, J. W., Kwon, D.-N., & Kim, J.-H. (2014). Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against Gram-negative and Gram-positive bacteria. *Nanoscale research letters*, 9, 1-17.
- Huang, Z., Zhu, M., & Zhang, G. (2022). Structure identification and functional mechanism of natural active components: A special issue. *Foods*, 11(9), 1285.
- Ibrahim, S. R., Mohamed, G. A., Khayat, M. T., Ahmed, S., Abo-Haded, H., & Alshali, K. Z. (2019). Mangostanaxanthone VIII, a new xanthone from *Garcinia mangostana* pericarps, α -amylase inhibitory activity, and molecular docking studies. *Revista Brasileira de Farmacognosia*, 29, 206-212.

- Ilaboya, I. R., & Oturo, E. A. (2019). Simple to use Microsoft Excel template for estimating the parameters of some selected probability distribution model by method of L-Moment. *parameters, 1*, 1.
- Janardhanan, S., Mahendra, J., Girija, A. S., Mahendra, L., & Priyadharsini, V. (2017). Antimicrobial effects of *Garcinia mangostana* on cariogenic microorganisms. *Journal of clinical and diagnostic research: JCDR, 11*(1), ZC19.
- Ji, B.-K., Gao, X.-M., Cui, D., Wang, S.-S., Huang, W.-Z., Li, Y.-K., Mei, S.-X., Yang, Z., Li, G.-P., & Jiang, M.-Y. (2017). Two new biphenyls from the stems of *Garcinia tetralata*. *Natural product research, 31*(13), 1544-1550.
- Joana, A., Fernandes, C., Pinto, M., & Tiritan, M. E. (2019). Chiral derivatives of xanthenes with antimicrobial activity. *Molecules, 24*(2), 314.
- Junior, M. A. D., Nguema Edzang, R. W., Catto, A. L., & Raimundo, J.-M. (2022). Quinones as an efficient molecular scaffold in the antibacterial/antifungal or antitumoral arsenal. *International Journal of Molecular Sciences, 23*(22), 14108.
- Kamal, N., Clements, C., Gray, A. I., & Edrada-Ebel, R. (2016). Anti-infective activities of secondary metabolites from *Vitex pinnata*. *Journal of Applied Pharmaceutical Science, 6*(1), 102-106.
- Karunakaran, T., Ee, G. C. L., Ismail, I. S., Mohd Nor, S. M., & Zamakshshari, N. H. (2018). Acetyl-and O-alkyl-derivatives of β -mangostin from *Garcinia mangostana* and their anti-inflammatory activities. *Natural product research, 32*(12), 1390-1394.
- Karunakaran, T., Firouz, N. S., Santhanam, R., & Jong, V. Y. M. (2022). Phytochemicals from *Calophyllum macrocarpum* Hook. f. and its cytotoxic activities. *Natural product research, 36*(2), 654-659.

- Keita, K., Darkoh, C., & Okafor, F. (2022). Secondary plant metabolites as potent drug candidates against antimicrobial-resistant pathogens. *SN Applied Sciences*, 4(8), 209.
- Khamidullina, E., Purevdash, M., Ushakov, I., Neretina, O., & Medvedeva, S. (2005). Phenolic compounds from *Pinus sibirica* shells. *Chemistry of natural compounds*, 41, 101-102.
- Khamthong, N., & Hutadilok-Towatana, N. (2017). Phytoconstituents and Biological Activities of *Garcinia dulcis* (Clusiaceae): A Review. *Natural Product Communications*, 12(3), 453-460.
- Koh, J.-J., Lin, S., Aung, T. T., Lim, F., Zou, H., Bai, Y., Li, J., Lin, H., Pang, L. M., & Koh, W. L. (2015). Amino acid modified xanthone derivatives: novel, highly promising membrane-active antimicrobials for multidrug-resistant Gram-positive bacterial infections. *Journal of medicinal chemistry*, 58(2), 739-752.
- Kuok, C. F., Hoi, S. O., Hoi, C. F., Chan, C. H., Fong, I. H., Ngok, C. K., Meng, L. R., & Fong, P. (2017). Synergistic antibacterial effects of herbal extracts and antibiotics on methicillin-resistant *Staphylococcus aureus*: A computational and experimental study. *Experimental Biology and Medicine (Maywood)*, 242(7), 731-743.
- Lannang, A. M., Sema, D. K., Tatsimo, S. J., Tankeu, V. T., Tegha, H. F., Wansi, J. D., Shiono, Y., & Sewald, N. (2018). A new depsidone derivative from the leaves of *Garcinia polyantha*. *Natural product research*, 32(9), 1033-1038.
- Larsuprom, L., Rungroj, N., Lekcharoensuk, C., Pruksakorn, C., Kongkiatpaiboon, S., Chen, C., & Sukatta, U. (2019). In vitro antibacterial activity of mangosteen (*Garcinia mangostana* Linn.) crude extract against *Staphylococcus pseudintermedius* isolates from canine pyoderma. *Veterinary dermatology*, 30(6), 487-e145.

- Lee, K. W., Zamakshshari, N. H., Ee, G. C. L., Mah, S. H., & Mohd Nor, S. M. (2018). Isolation and structural modifications of ananixanthone from *Calophyllum teysmannii* and their cytotoxic activities. *Natural product research*, 32(18), 2147-2151.
- Lim, S. H., Lee, H. S., Lee, C. H., & Choi, C.-I. (2021). Pharmacological activity of *Garcinia indica* (Kokum): An updated review. *Pharmaceuticals*, 14(12), 1338.
- Liu, X., Shen, J., & Zhu, K. (2022). Antibacterial activities of plant-derived xanthenes. *RSC Med Chem*, 13(2), 107-116.
- Mahamodo, S., Rivière, C., Neut, C., Abedini, A., Ranarivelo, H., Duhal, N., Roumy, V., Hennebelle, T., Sahpaz, S., & Lemoine, A. (2014). Antimicrobial prenylated benzoylphloroglucinol derivatives and xanthenes from the leaves of *Garcinia goudotiana*. *Phytochemistry*, 102, 162-168.
- Mahizan, N. A., Yang, S.-K., Moo, C.-L., Song, A. A.-L., Chong, C.-M., Chong, C.-W., Abushelaibi, A., Lim, S.-H. E., & Lai, K.-S. (2019). Terpene derivatives as a potential agent against antimicrobial resistance (AMR) pathogens. *Molecules*, 24(14), 2631.
- Mañourová, A., Leuner, O., Tchoundjeu, Z., Van Damme, P., Verner, V., Příbyl, O., & Lojka, B. (2019). Medicinal potential, utilization and domestication status of bitter kola (*Garcinia kola* Heckel) in West and Central Africa. *Forests*, 10(2), 124.
- Marianna, O. A., Freire Pessoa, H. L., Lira, A. B., Castillo, Y. P., & de Sousa, D. P. (2019). Synthesis, antibacterial evaluation, and QSAR of caffeic acid derivatives. *Journal of Chemistry*, 2019, 3408315.
- Mitschke, N., Vemulapalli, S., & Dittmar, T. (2023). NMR spectroscopy of dissolved organic matter: a review. *Environmental Chemistry Letters*, 21(2), 689-723.

- Muhammad, N. A., Basar, N., & Jamil, S. (2019). Antibacterial activity of phytochemicals from *garcinia parvifolia* miq. and *garcinia hombroniana* pierre. *Journal of Science and Mathematics Letters*, 7, 44-51.
- Murmu, P., Kumar, S., Patra, J. K., Singh, N., Rath, S., Chan, & Yean, Y. (2016). Ethnobotanical, nutritional, phytochemical and antimicrobial studies of *garcinia xanthochymus* fruit extracts. *British Biotechnology Journal*, 13, 25244.
- Murthy, H. N., Dalawai, D., Dewir, Y. H., & Ibrahim, A. (2020). Phytochemicals and biological activities of *Garcinia morella* (Gaertn.) Desr.: A review. *Molecules*, 25(23), 5690.
- Musa, N. F., Azam, Z. M., Shadan, N. H., & Zalina, N. (2020). Evaluation of the antioxidant and antimicrobial activities of leaves and fruits extracts from *Garcinia atroviridis*. 3rd ICA Research Symposium (ICARS) 2020 (p.44).
- Naves, V. M. L., dos Santos, M. H., Ribeiro, I. S., da Silva, C. A., Silva, N. C., da Silva, M. A., da Silva, G. A., Dias, A. L. T., Ionta, M., & Dias, D. F. (2019). Antimicrobial and antioxidant activity of *Garcinia brasiliensis* extracts. *South African Journal of Botany*, 124, 244-250.
- Ngoupayo, J., Tabopda, T. K., & Ali, M. S. (2009). Antimicrobial and immunomodulatory properties of prenylated xanthenes from twigs of *Garcinia staudtii*. *Bioorganic & medicinal chemistry*, 17(15), 5688-5695.
- Nguyen, L.-H. D., Vo, H. T., Pham, H. D., Connolly, J. D., & Harrison, L. J. (2003). Xanthenes from the bark of *Garcinia merguensis*. *Phytochemistry*, 63(4), 467-470.

- Nguyen, N. H., Nguyen, M. T., Nguyen, H. D., Pham, P. D., Thach, U. D., Trinh, B. T., Nguyen, L. T., Dang, S. V., Do, A. T., & Do, B. H. (2021). Antioxidant and antimicrobial activities of the extracts from different *garcinia* species. *Evidence-Based Complementary and Alternative Medicine*, 2021, 5542938.
- Novakovic, M., Battistel, M. D., Azurmendi, H. F., Concilio, M.-G., Freedberg, D. I., & Frydman, L. (2021). The incorporation of labile protons into multidimensional nmr analyses: glycan structures revisited. *Journal of the American Chemical Society*, 143(23), 8935-8948.
- Nurakmal, M., Rahman, A., Nafiah, M., Mohd, W., Hakimi, N., Salleh, W., Tan, S.-P., Mohd Hashim, N., & Zamakshshari, N. (2022). Antioxidant, antimicrobial, and cytotoxic activities of the hexane and dichloromethane extracts of Malaysian *Mitragyna speciosa* Korth. Leaves. *Malaysian Journal of Chemistry*, 24, 191-198.
- Nurul, C. H., Taher, M., & Susanti, D. (2018). Phytochemical constituents and pharmacological properties of *Garcinia xanthochymus*-a review. *Biomedicine & Pharmacotherapy= Biomedecine & Pharmacotherapie*, 106, 1378-1389.
- Pailee, P., Kuhakarn, C., Sangsuwan, C., Hongthong, S., Piyachaturawat, P., Suksen, K., Jariyawat, S., Akkarawongsapat, R., Limthongkul, J., & Napaswad, C. (2018). Anti-HIV and cytotoxic biphenyls, benzophenones and xanthenes from stems, leaves and twigs of *Garcinia speciosa*. *Phytochemistry*, 147, 68-79.
- Paul, A., & Zaman, M. K. (2022). A comprehensive review on ethnobotany, nutritional values, phytochemistry and pharmacological attributes of ten potent *Garcinia* species of South-east Asia. *South African Journal of Botany*, 148, 39-59.

- Pedraza-Chaverri, J., Cárdenas-Rodríguez, N., Orozco-Ibarra, M., & Pérez-Rojas, J. M. (2008). Medicinal properties of mangosteen (*Garcinia mangostana*). *Food and chemical toxicology*, 46(10), 3227-3239.
- Perry, E. K., Meirelles, L. A., & Newman, D. K. (2022). From the soil to the clinic: the impact of microbial secondary metabolites on antibiotic tolerance and resistance. *Nature Reviews Microbiology*, 20(3), 129-142.
- Piechota, M., Kot, B., Frankowska-Maciejewska, A., Grużewska, A., & Woźniak-Kosek, A. (2018). Biofilm formation by methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* strains from hospitalized patients in Poland. *BioMed research international*, 2018(1), 4657396.
- Porras, G., Chassagne, F., Lyles, J. T., Marquez, L., Dettweiler, M., Salam, A. M., Samarakoon, T., Shabih, S., Farrokhi, D. R., & Quave, C. L. (2021). Ethnobotany and the role of plant natural products in antibiotic drug discovery. *Chemical Reviews*, 121(6), 3495-3560.
- Plants of the World Online. (2024). Facilitated by the royal botanic gardens, Kew. Available at: <http://www.plantsoftheworldonline.org/> Assessed on 16 March 2024.
- Raksat, A., Maneerat, W., Andersen, R. J., Pyne, S. G., & Laphookhieo, S. (2019). A tocotrienol quinone dimer and xanthones from the leaf extract of *Garcinia nigrolineata*. *Fitoterapia*, 136, 104175.
- Raksat, A., Phukhatmuen, P., Yang, J., Maneerat, W., Charoensup, R., Andersen, R. J., Wang, Y. A., Pyne, S. G., & Laphookhieo, S. (2020). Phloroglucinol benzophenones and xanthones from the leaves of *Garcinia cowa* and their nitric oxide production and α -glucosidase inhibitory activities. *Journal of Natural Products*, 83(1), 164-168.

- Reygaert, W. C. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS microbiology*, 4(3), 482.
- Ruan, J., Zheng, C., Liu, Y., Qu, L., Yu, H., Han, L., Zhang, Y., & Wang, T. (2017). Chemical and biological research on herbal medicines rich in xanthones. *Molecules*, 22(10), 1698.
- Saepudin, A., Natawijaya, D., Hartini, E., & Iskandar, R. (2019). Evaluation of antibacterial activity of mangosteen (*Garcinia mangostana* L.) pericarp extract against rice leaf blight bacteria (*Xanthomonas oryzae* pv. *oryzae*) at various temperatures and durations of fruit storage. IOP Conference Series: Earth and Environmental Science (Vol. 250, No. 1, p.012026).
- Sahariah, B., & Sarma, B. K. (2020). Spectroscopic evidence of $n \rightarrow \pi^*$ interactions involving carbonyl groups. *Physical Chemistry Chemical Physics*, 22(46), 26669-26681.
- Santo, B. L. S. d. E., Santana, L. F., Kato Junior, W. H., de Araújo, F. d. O., Bogo, D., Freitas, K. d. C., Guimarães, R. d. C. A., Hiane, P. A., Pott, A., & Filiú, W. F. d. O. (2020). Medicinal potential of *Garcinia* species and their compounds. *Molecules*, 25(19), 4513.
- Santos, R. A., Pinto, D. C., & Silva, A. M. (2022). NMR structural characterization of oxygen heterocyclic compounds. *Heterocycles: Synthesis, Catalysis, Sustainability, and Characterization*, 453-524.
- See, I., Ee, G. C. L., Jong, V. Y. M., Teh, S. S., Acuña, C. L. C., & Mah, S. H. (2021). Cytotoxic activity of phytochemicals from *Garcinia mangostana* L. and *G. benthamiana* (Planch. & Triana) Pipoly against breast cancer cells. *Natural product research*, 35(24), 6184-6189.

- See, I., Ee, G. C. L., Teh, S. S., Mah, S. H., Karjiban, R. A., Daud, S., & Jong, V. Y. M. (2016). A new benzophenone from *Garcinia benthamiana*. *Records of Natural Products*, 10(3), 355.
- Shahid, M., Law, D., Azfaralariff, A., Mackeen, M. M., Chong, T. F., & Fazry, S. (2022). Phytochemicals and biological activities of *garcinia atroviridis*: A critical review. *Toxics*, 10(11), 656.
- Silva, R. E. d., Ribeiro, F. d. O. S., de Carvalho, A. M. A., Daboit, T. C., Marinho-Filho, J. D. B., Matos, T. S., Pessoa, O. D. L., de Almeida, J. R. d. S., de Araújo, A. R., & dos Santos Soares, M. J. (2020). Antimicrobial and antibiofilm activity of the benzoquinone oncocalyxone A. *Microbial Pathogenesis*, 149, 104513.
- Simpson, J. H. (2017). *NMR case studies: data analysis of complicated molecules*. Elsevier.
- Sukandar, E. R., Kaennakam, S., Aree, T., Nöst, X., Rassamee, K., Bauer, R., Siripong, P., Ersam, T., & Tip-Pyang, S. (2020). Picrorhizones A–H, polyprenylated benzoylphloroglucinols from the stem bark of *Garcinia picrorhiza*. *Journal of Natural Products*, 83(7), 2102-2111.
- Sukandar, E. R., Kaennakam, S., Rassamee, K., Ersam, T., Siripong, P., & Tip-Pyang, S. (2019). Tetrandraxanthones A–I, prenylated and geranylated xanthones from the stem bark of *Garcinia tetrandra*. *Journal of Natural Products*, 82(5), 1312-1318.
- Sultan, O. S., Kantilal, H. K., Khoo, S. P., Davamani, A. F., Eusufzai, S. Z., Rashid, F., Jamayet, N. B., Soh, J. A., Tan, Y. Y., & Alam, M. K. (2022). The Potential of α -mangostin from *Garcinia mangostana* as an effective antimicrobial agent—a systematic review and meta-analysis. *Antibiotics*, 11(6), 717.

- Taher, M., Idris, M. S., & Arbain, D. (2007). Antimicrobial, antioxidant, anti-inflammatory and cytotoxic activities of *Garcinia eugenifolia* and *Calophyllum enervosum*. *Iranian Journal of Pharmacology & Therapeutics*, 6(1), 93-98.
- Tanha, F. A. (2017). *Antioxidant and anti-microbial activity assessment of methanol extract of Garcinia cowa leaves*. East West University.
- Thuy, T. T. T., Nguyet, H. T. M., Thuy, N. P., Vu, N. N., Binh, N. Q., Huu, B., & Giang, P. M. (2022). Chemical constituents of dichloromethane extract from the stem bark of *Garcinia fagraeoides*. *Vietnam Journal of Science and Technology*, 60(6), 958-965.
- Trinh, D. H., Huynh, O. N., Nguyen, T. Q., Nguyen, H. T., Nguyen, T.-L. T., Pham, H. D., Nguyen, L.-H. D., & Tran, P. T. (2023). Polyisoprenylated benzophenones and xanthenes from the pericarp of *Garcinia planchonii* Pierre. *Natural product research*, 37(2), 188-196.
- Velu, G., Palanichamy, V., & Rajan, A. P. (2018). Phytochemical and pharmacological importance of plant secondary metabolites in modern medicine. *Bioorganic phase in natural food: an overview*, 135-156.
- Verheijen, M., Lienhard, M., Schrooders, Y., Clayton, O., Nudischer, R., Boerno, S., Timmermann, B., Selevsek, N., Schlapbach, R., Gmuender, H., Gotta, S., Geraedts, J., Herwig, R., Kleinjans, J., & Caiment, F. (2019). DMSO induces drastic changes in human cellular processes and epigenetic landscape in vitro. *Scientific Reports*, 9(1), 4641.
- Waksmundzka-Hajnos, M., Sherma, J., & Kowalska, T. (2008). *Thin layer chromatography in phytochemistry*. CRC Press.

- Wang, W., Liao, Y., Huang, X., Tang, C., & Cai, P. (2018). A novel xanthone dimer derivative with antibacterial activity isolated from the bark of *Garcinia mangostana*. *Natural product research*, 32(15), 1769-1774.
- Wong, K. W., Ee, G. C. L., Ismail, I. S., Karunakaran, T., & Jong, V. Y. M. (2017). A new pyranoxanthone from *Garcinia nervosa*. *Natural product research*, 31(21), 2513-2519.
- Yan, Y., Li, X., Zhang, C., Lv, L., Gao, B., & Li, M. (2021). Research progress on antibacterial activities and mechanisms of natural alkaloids: A review. *Antibiotics (Basel)*, 10(3).
- Yao, H., Liu, J., Xu, S., Zhu, Z., & Xu, J. (2017). The structural modification of natural products for novel drug discovery. *Expert opinion on drug discovery*, 12(2), 121-140.
- Yuanita, E., Jannah, B. K., Ulfa, M., Ningsih, B. N. S., & Dharmayani, N. K. T. (2023). C-prenylation of 1, 3 dihydroxyxanthone: synthesis, characterization and antibacterial activity. *Acta Chimica Asiana*, 6(1), 279-286.
- Zamakshshari, N. H., Ahmed, I. A., Didik, N. A. M., Nasharuddin, M. N. A., Hashim, N. M., & Abdullah, R. (2022). Chemical profile and antimicrobial activity of essential oil and methanol extract from peels of four *Durio zibethinus* L. varieties. *Biomass Conversion and Biorefinery*, 1-9.
- Zhang, X., Bai, Y., Deng, J., Zhuang, P., & Wang, H. (2024). Effects of nonaromatic through-bond conjugation and through-space conjugation on the photoluminescence of nontraditional luminogens. *Aggregate*, 5(3), e517.

- Zhao, Y. J., Zhai, Y. Q., Ma, G. H., & Su, Z. G. (2009). Kinetic analysis and improvement of the Williamson reaction for the synthesis of poly (ethylene glycol) propionaldehyde. *Journal of Applied Polymer Science*, 111(3), 1638-1643.
- Zou, H., Koh, J.-J., Li, J., Qiu, S., Aung, T. T., Lin, H., Lakshminarayanan, R., Dai, X., Tang, C., & Lim, F. H. (2013). Design and synthesis of amphiphilic xanthone-based, membrane-targeting antimicrobials with improved membrane selectivity. *Journal of medicinal chemistry*, 56(6), 2359-2373.

APPENDICES

Appendix A: 2D-NMR Spectra of Rubraxanthone (2)

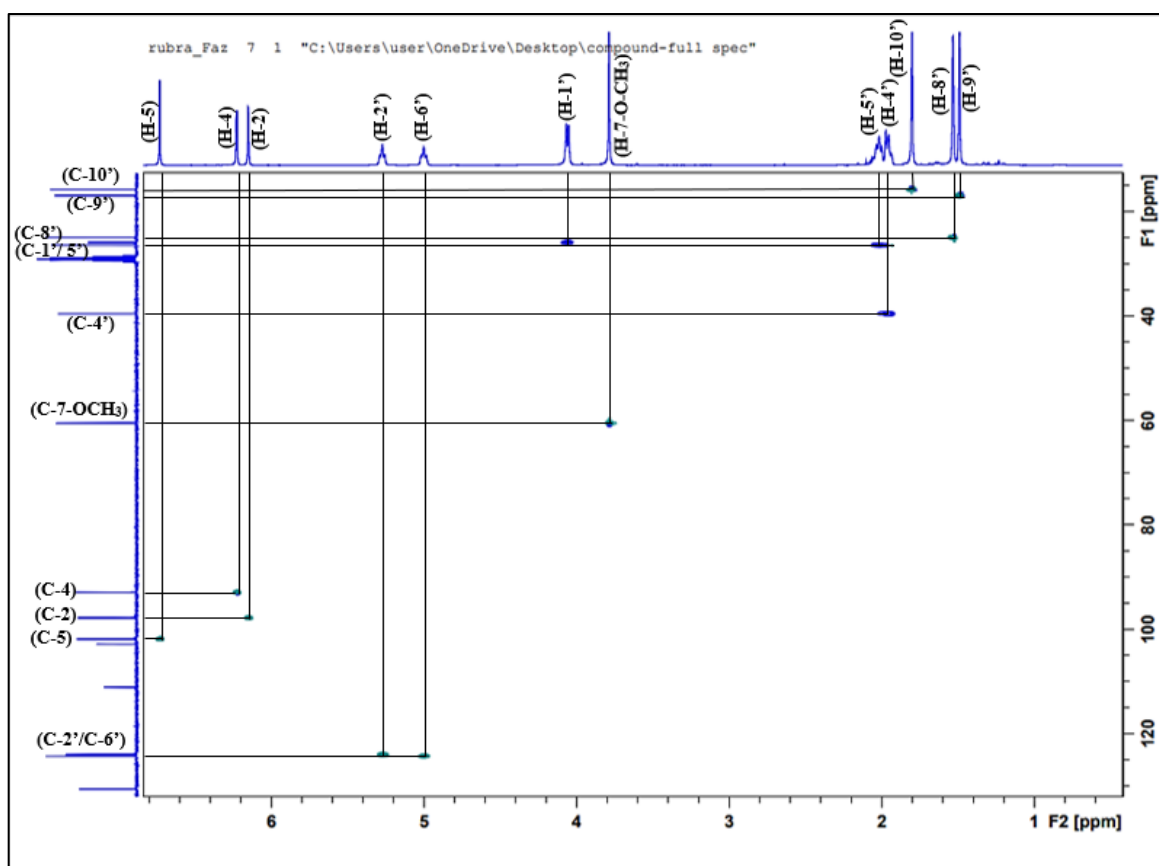
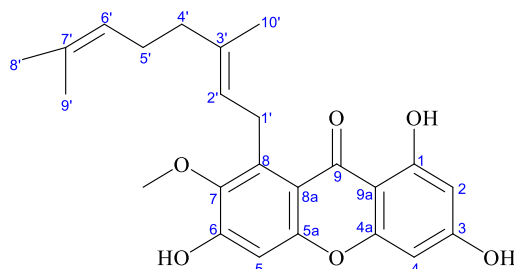


Figure 1: HSQC spectra of compound 2

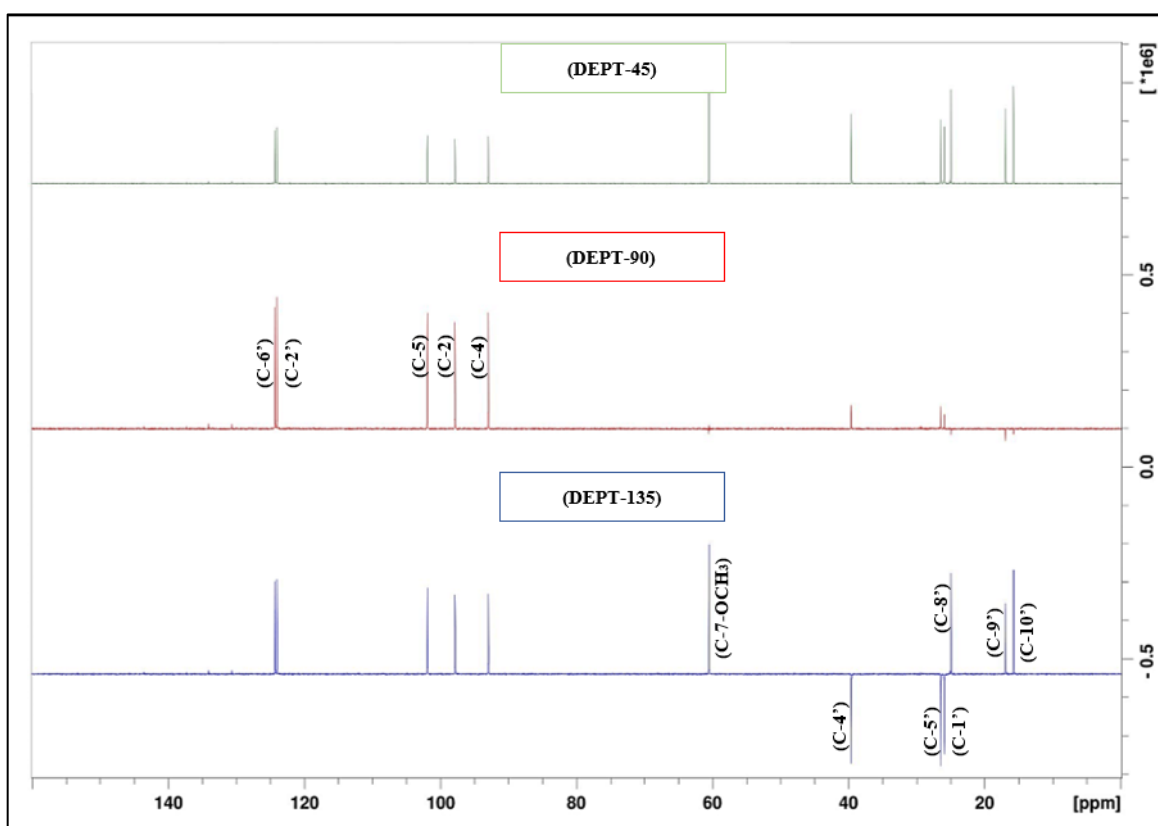
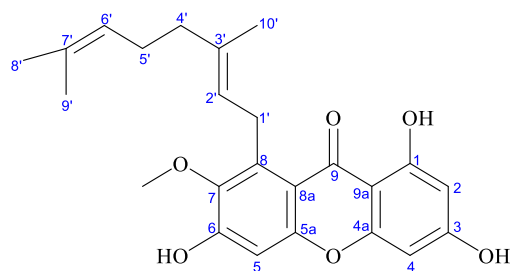


Figure 2: DEPT spectra of compound 2

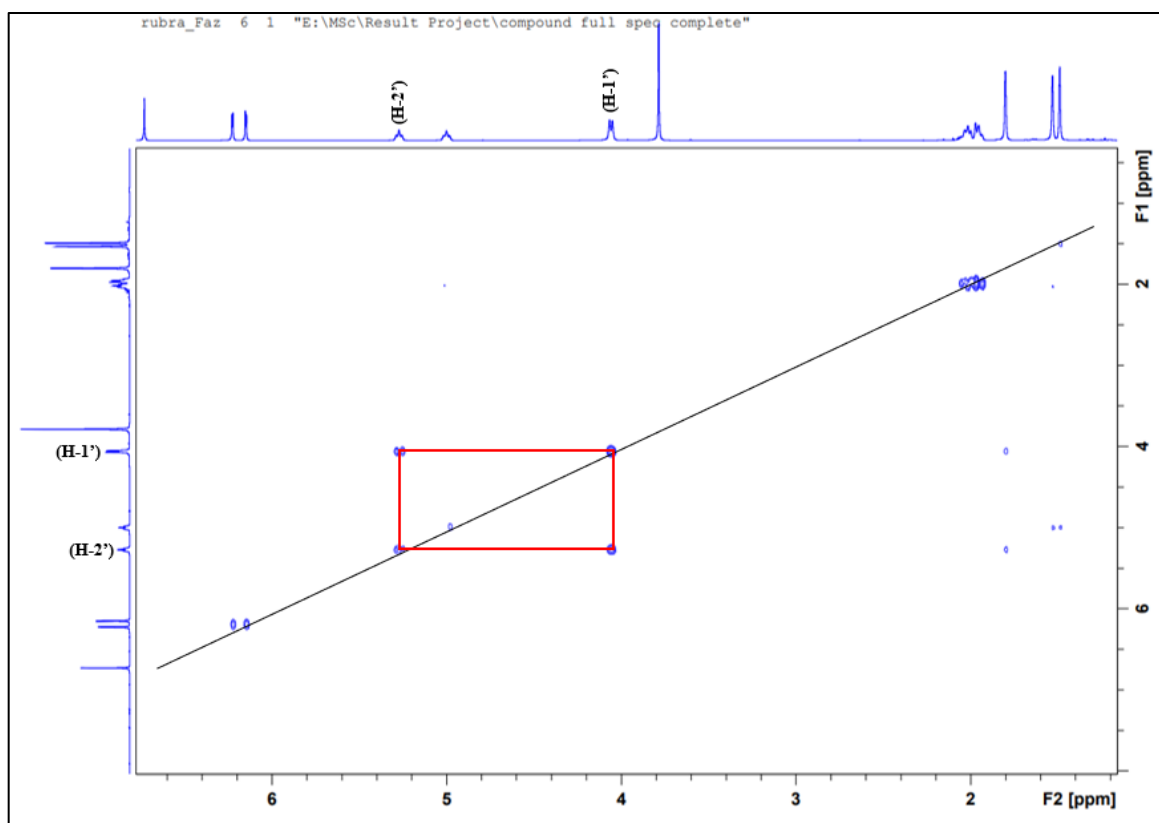
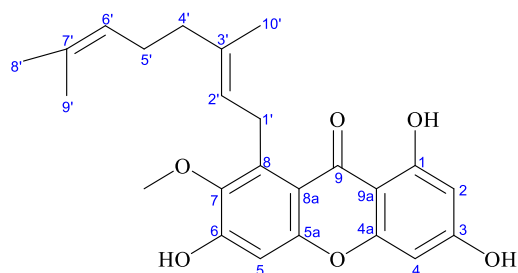


Figure 3: COSY spectra of compound 2

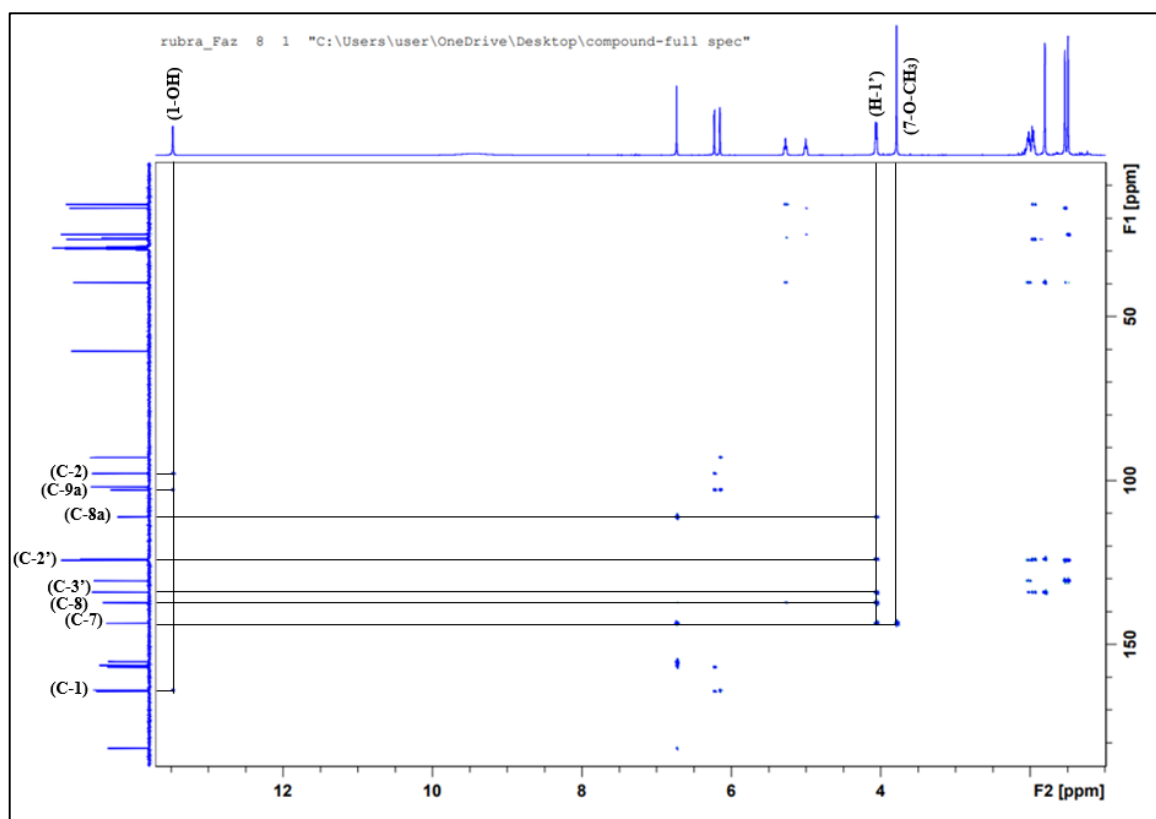
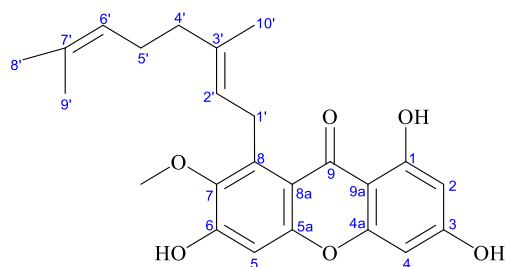


Figure 4: HMBC spectra of compound 2

Appendix B: 2D-NMR Spectra of 8-deoxygartanin (77)

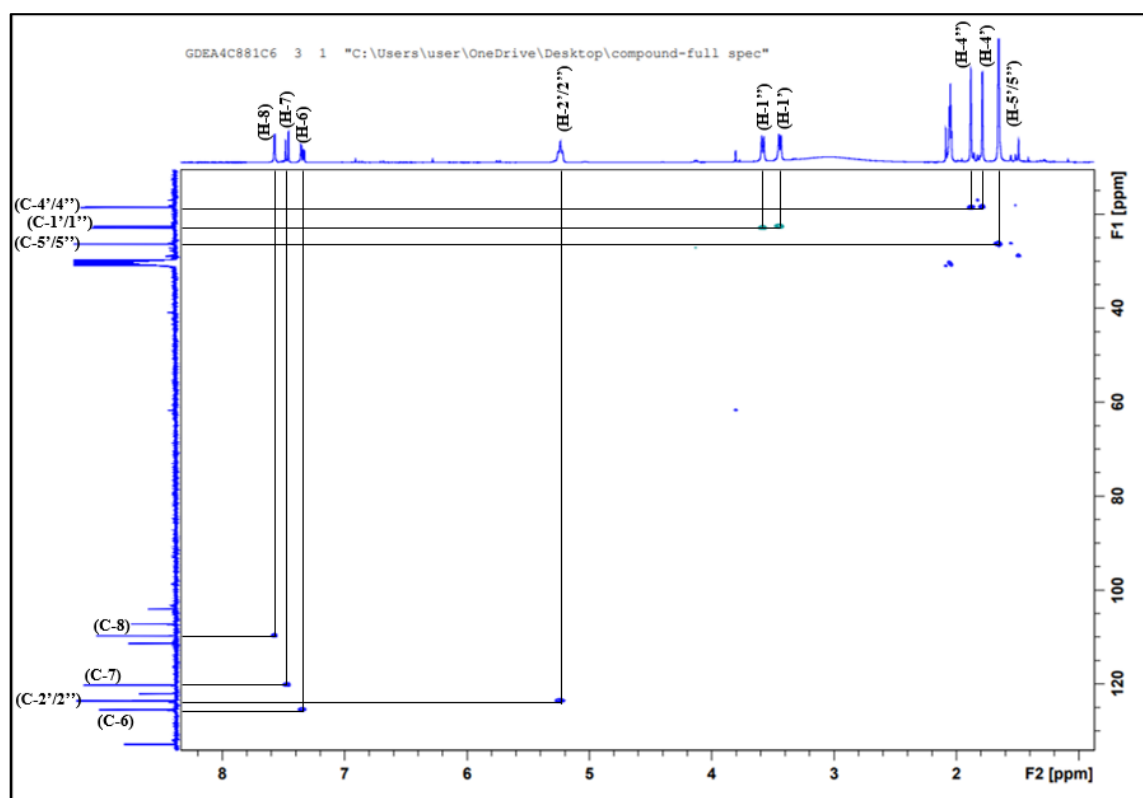
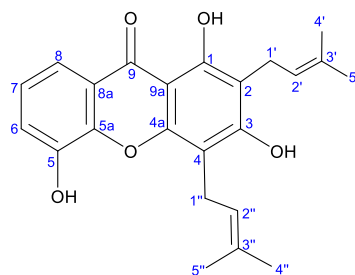


Figure 1: HSQC spectra of compound 77

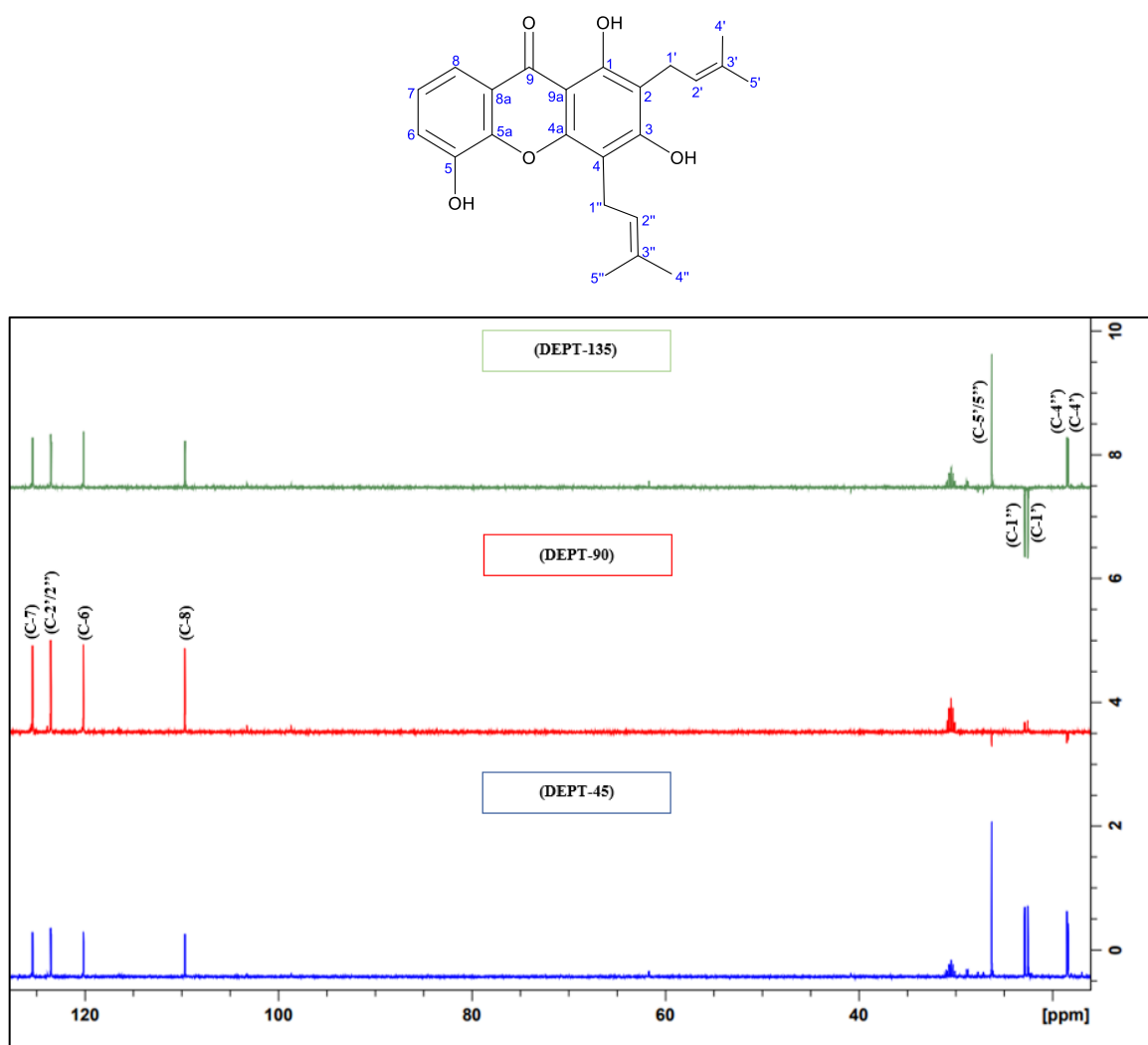


Figure 2: DEPT spectra of compound 77

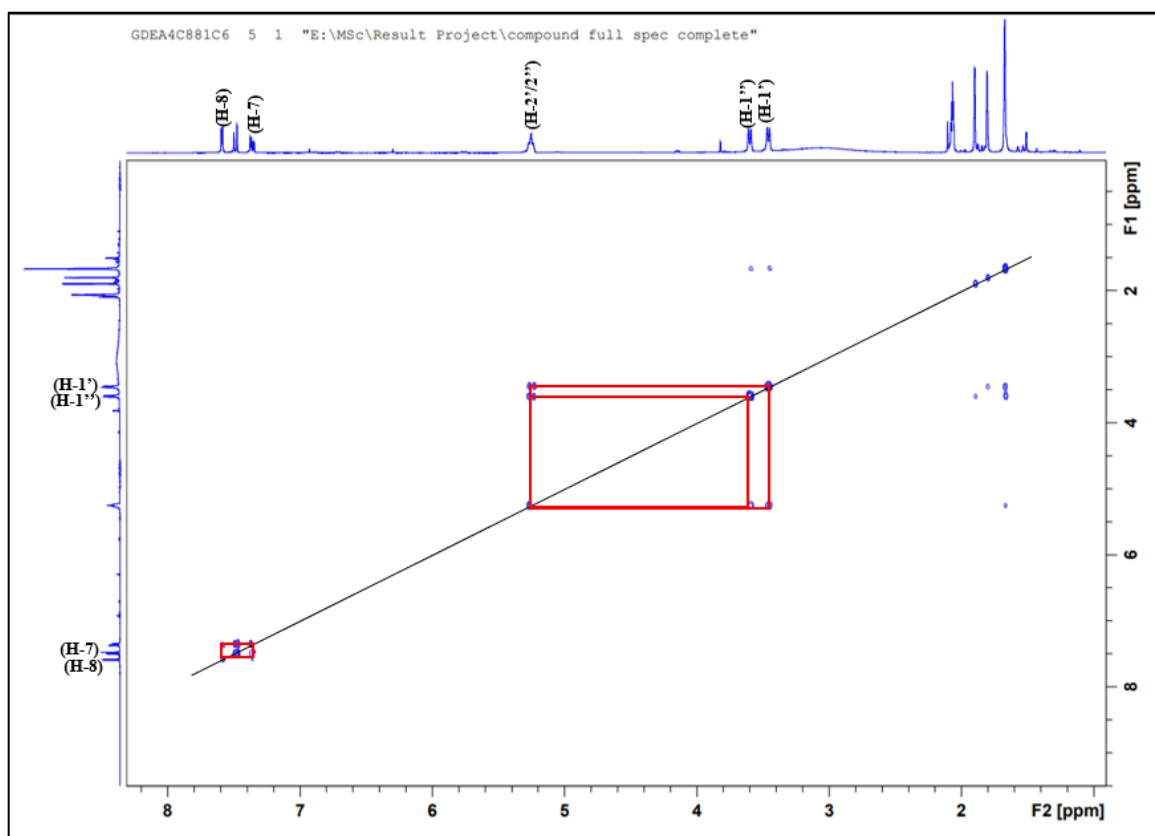
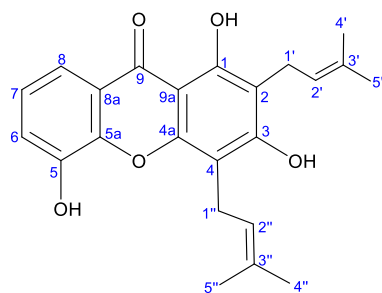


Figure 3: COSY spectra of compound 77

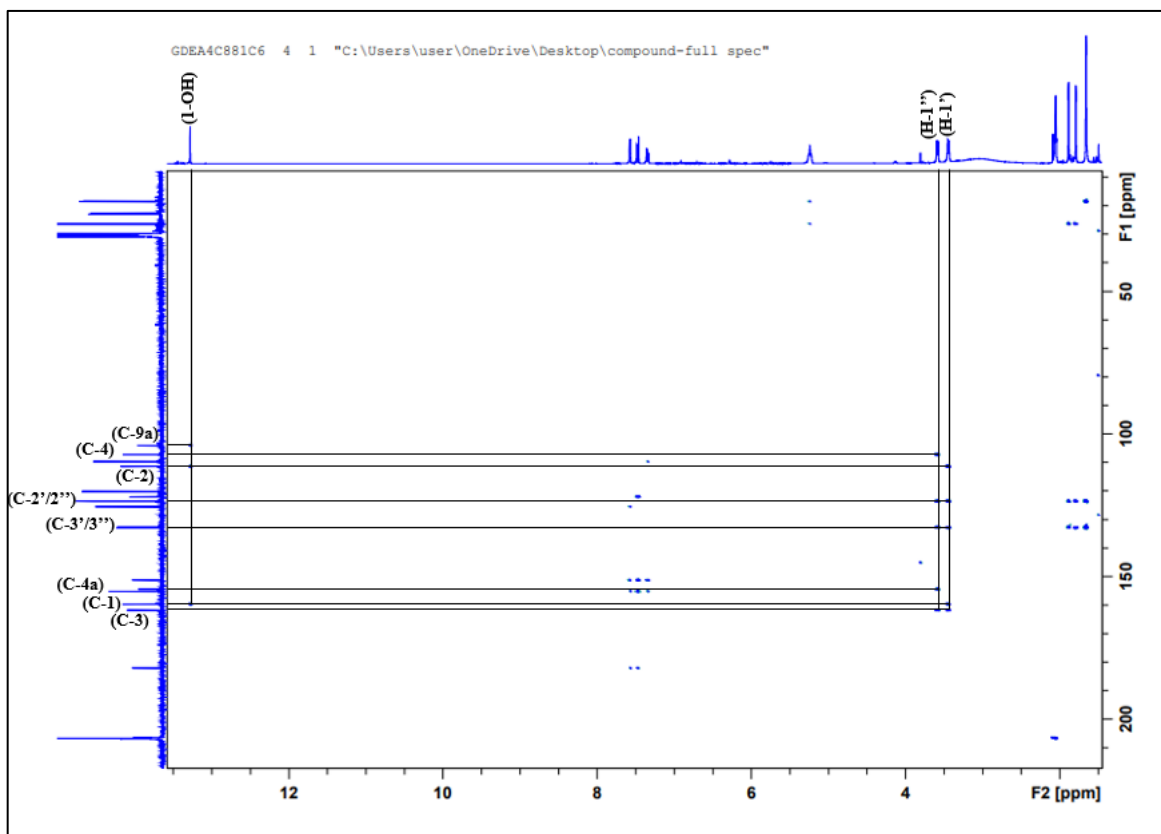
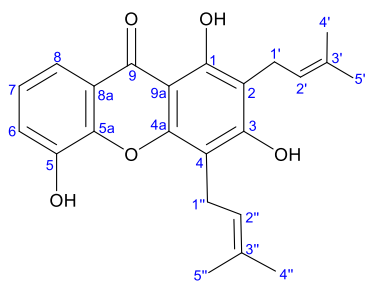


Figure 4: HMBC spectra of compound **77**

Appendix C: 2D-NMR Spectra of Isocowanol (78)

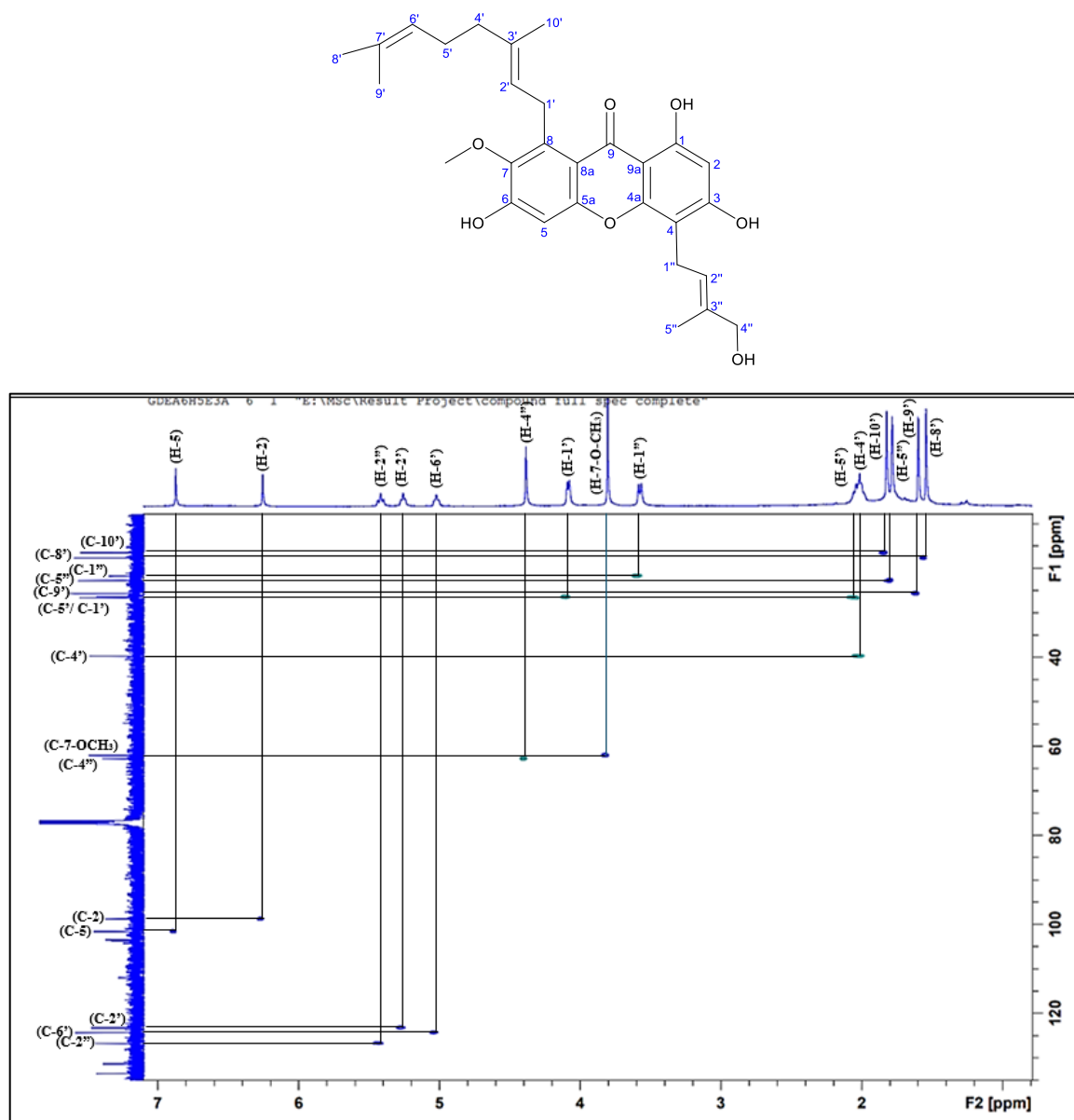


Figure 1: HSQC spectra of compound 78

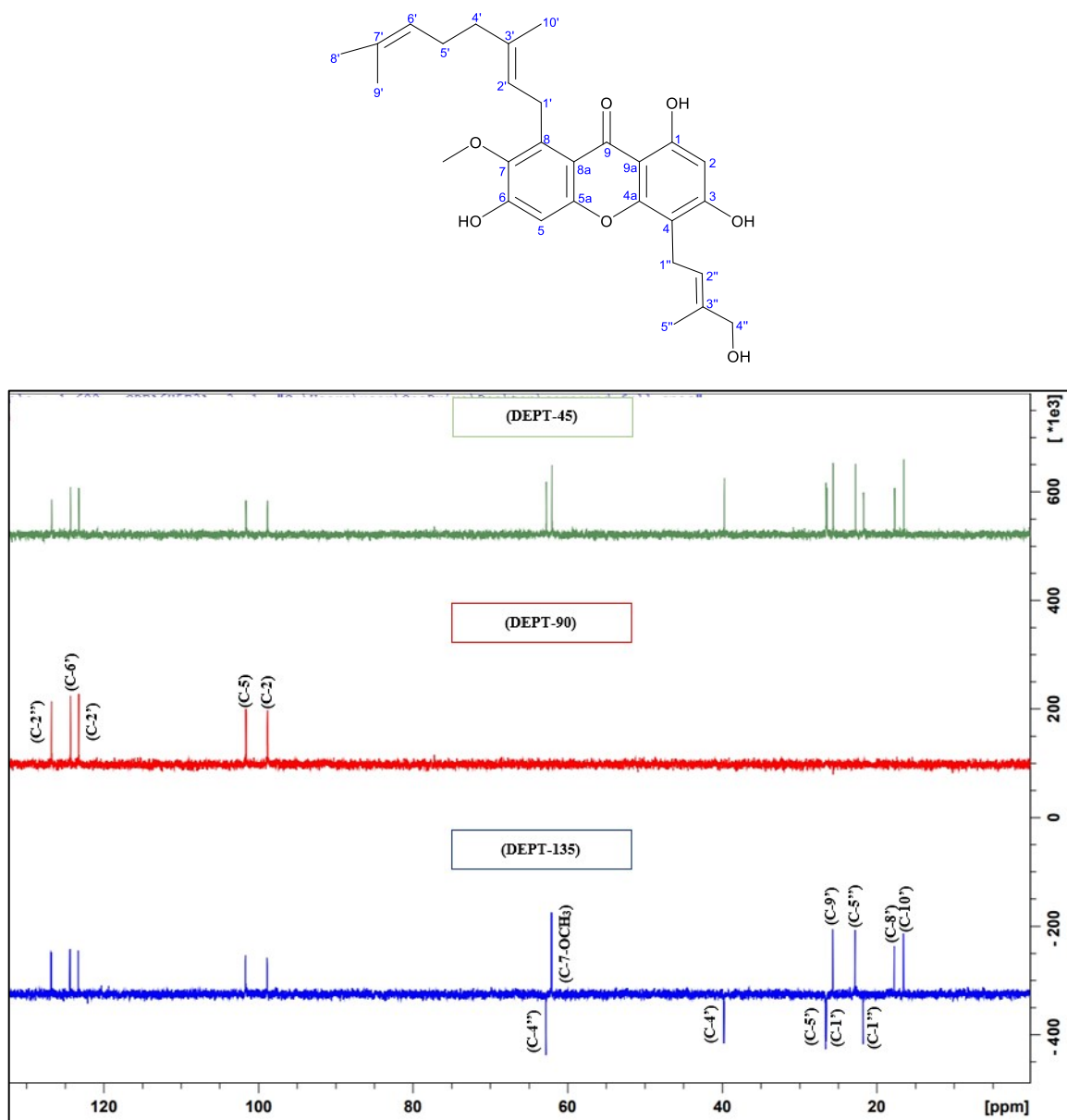
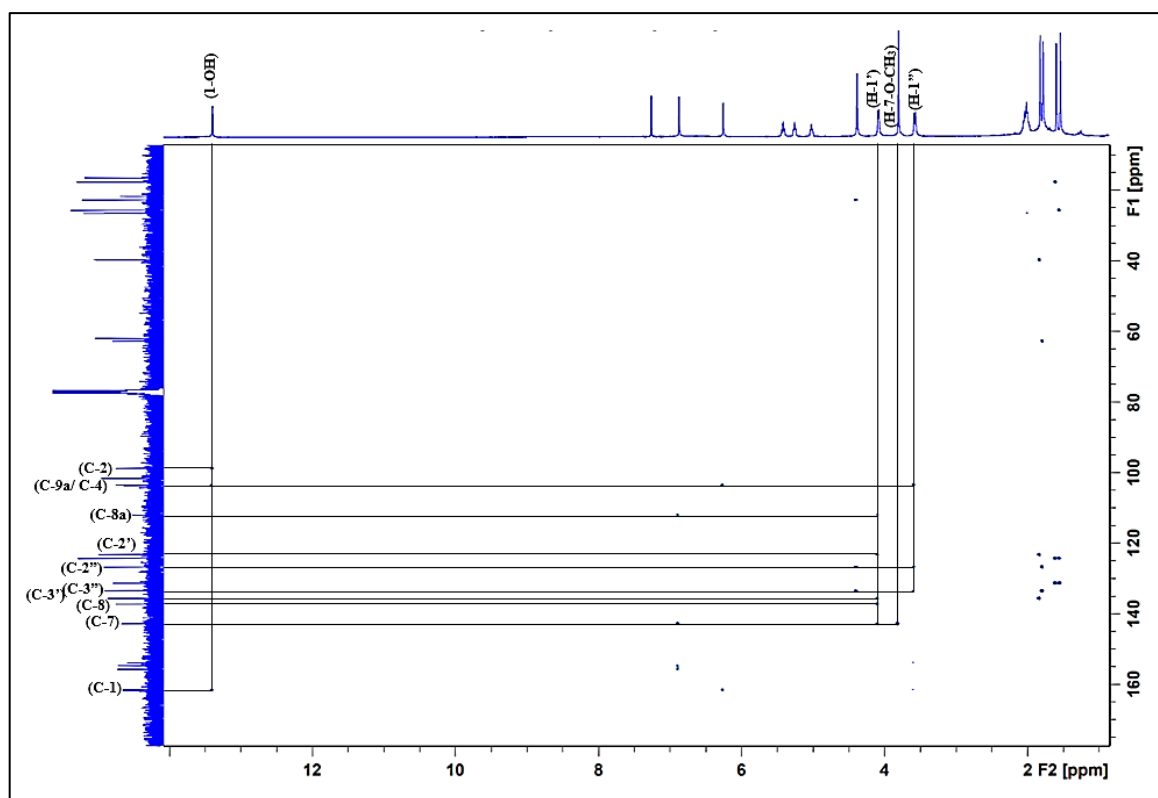


Figure 2: DEPT spectra of compound 78



150

Appendix D: 2D-NMR Spectra of 9-hydroxycalabaxanthone (79)

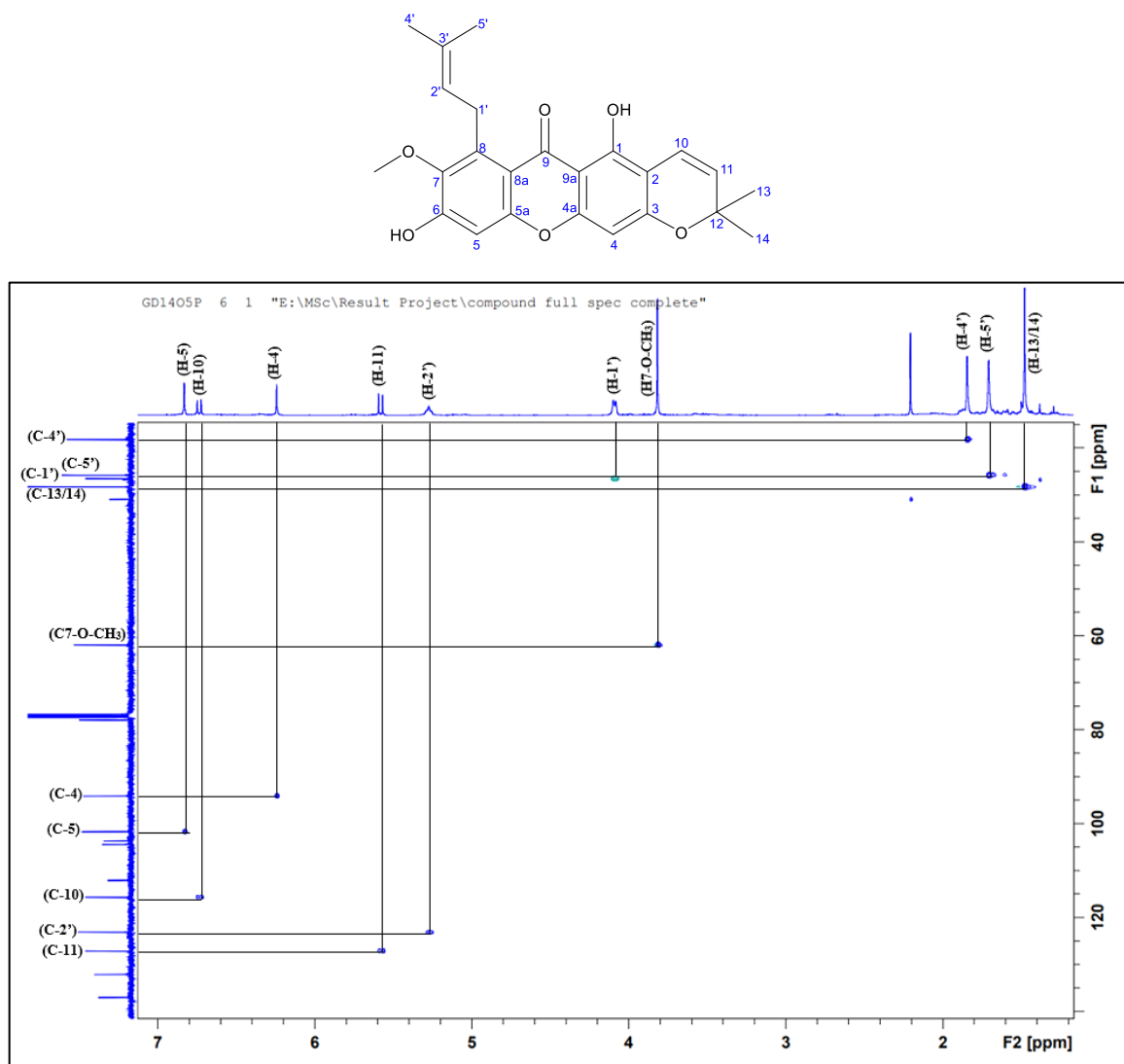


Figure 1: HSQC spectra of compound 79

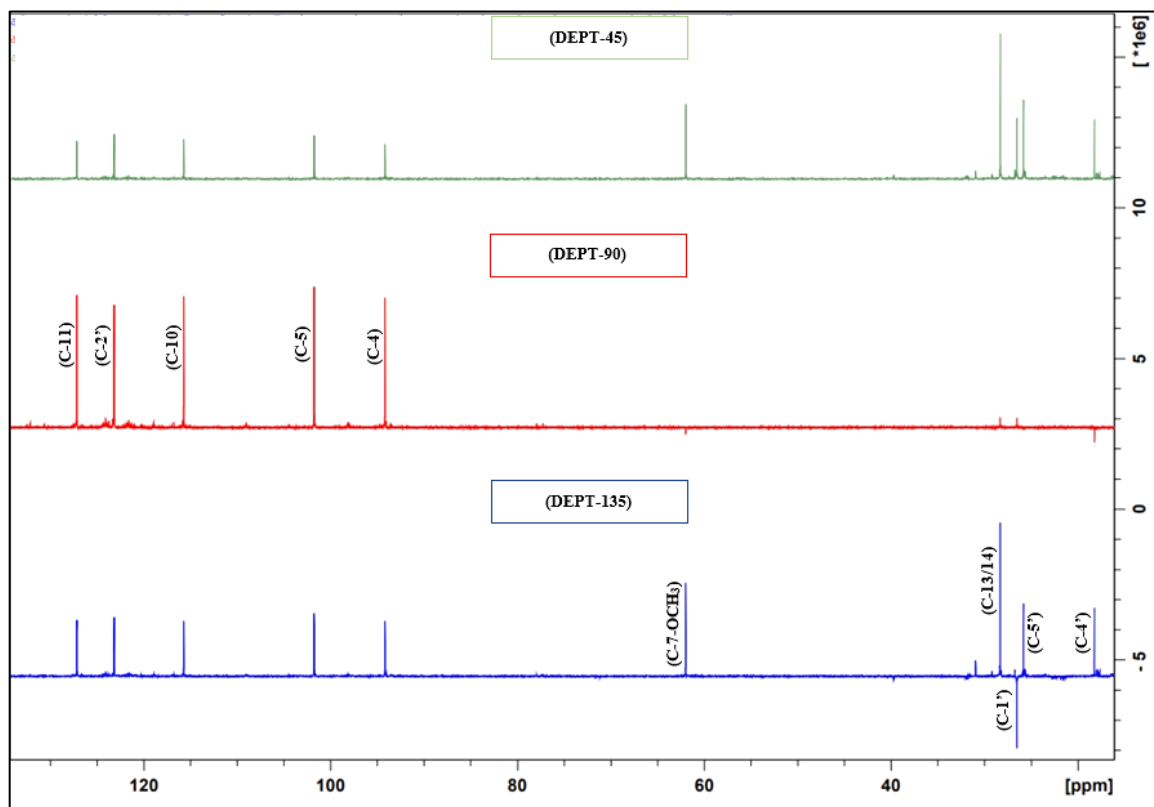
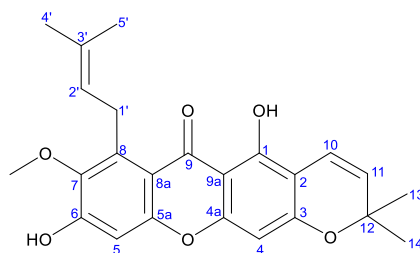


Figure 2: DEPT spectra of compound 79

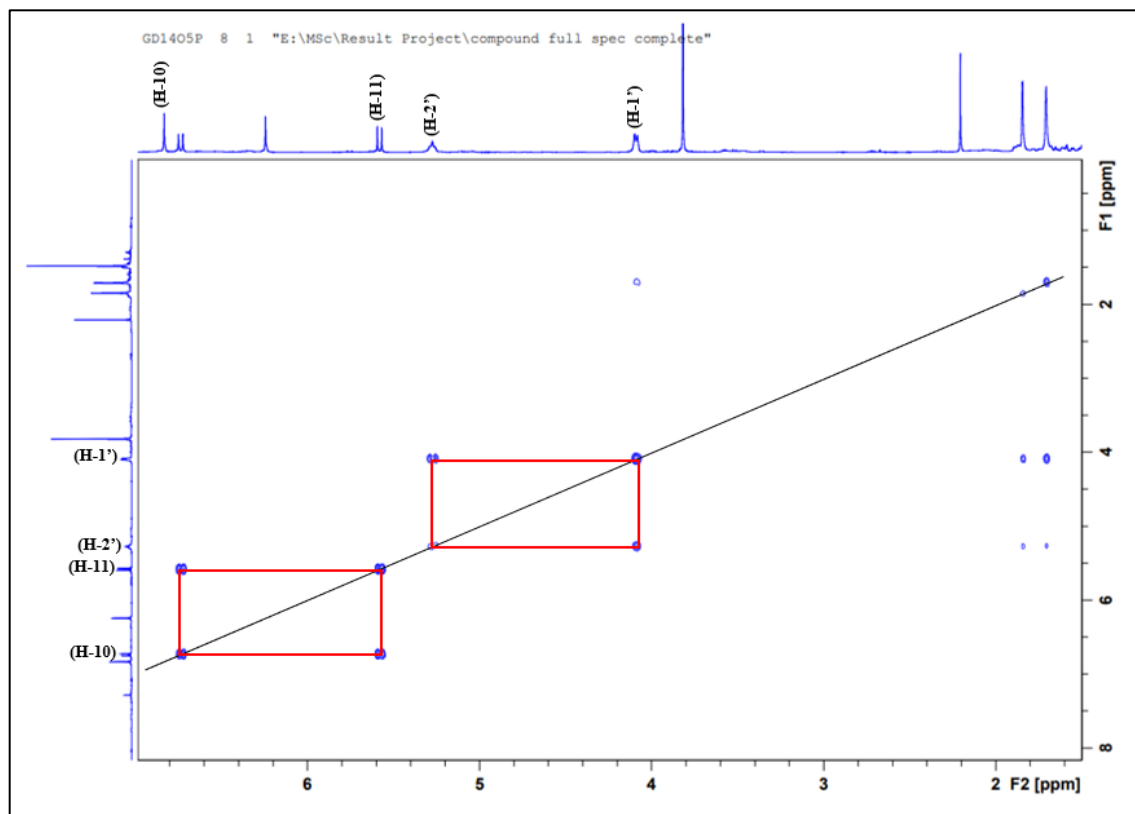
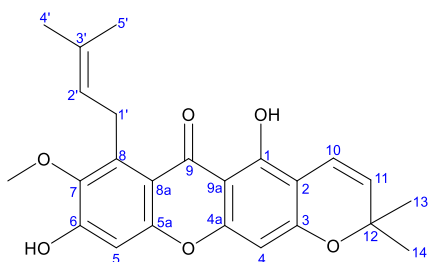


Figure 3: COSY spectra of compound **79**

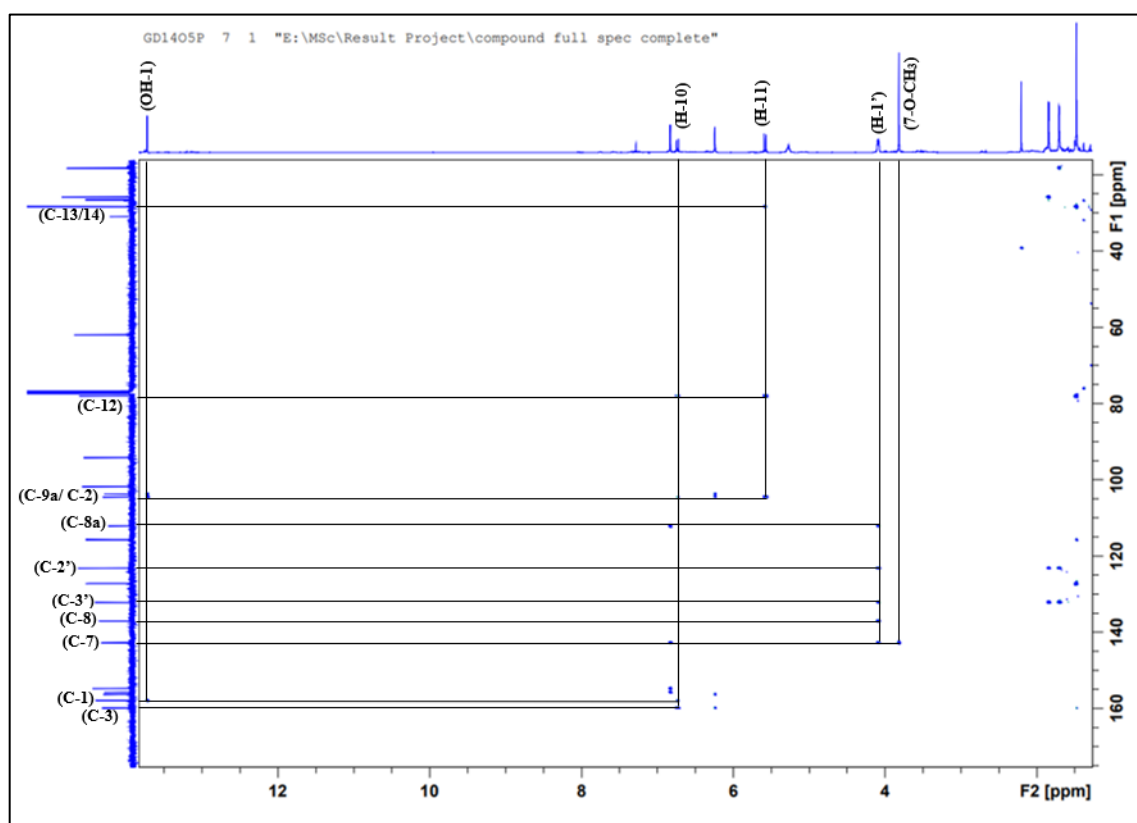
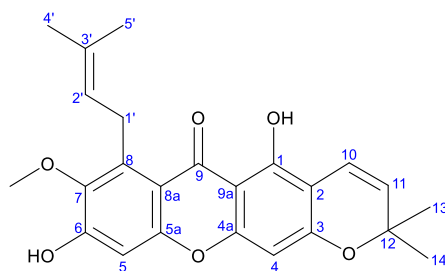


Figure 4: HMBC spectra of compound 79

Appendix E: Antibacterial Activity (Agar Well Diffusion Assay)



Figure 1: Inhibition zone by: AE) Ampicillin (1000 $\mu\text{g/mL}$); AC) Ampicillin (100 $\mu\text{g/mL}$); D) DMSO; H) GDH; EA) GDEA; M) GDM; C) 2-hydroxy-(4-octyloxy)benzophenone (**76**); R) rubraxanthone (**2**); 8D) 8-deoxygartanin (**77**); I) isocowanol (**78**); 9H) 9-hydroxycalabaxanthone (**79**); S) stigmasterol (**59**); 3T) 3-*O*-propyl-rubraxanthone (**80**); 3B) 6-*O*-propyl-rubraxanthone (**81**); C5) 3,6-*O*-pentyl-rubraxanthone (**82**); C6) 3,6-*O*-hexyl-rubraxanthone (**83**)

Appendix F: Antibacterial Activity (Minimum Inhibition Concentration)

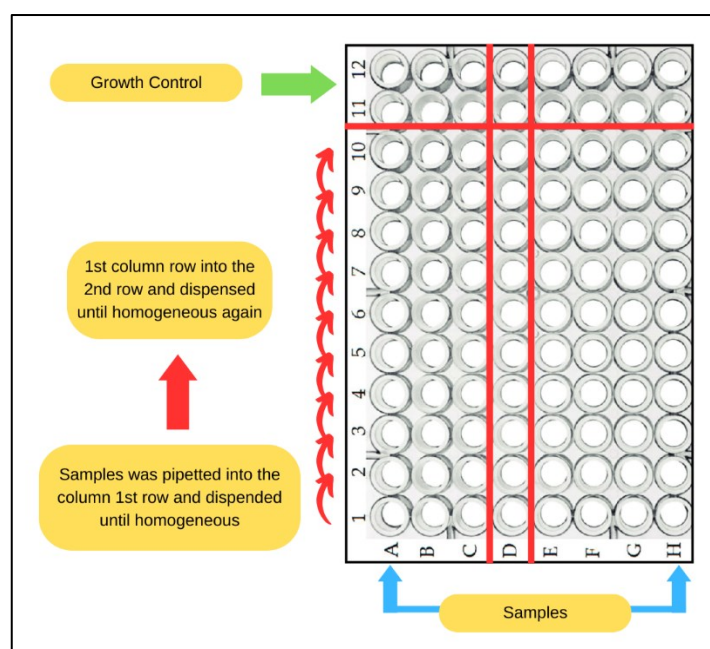


Figure 1: Example of a 96-well plate set up for determination of the MIC